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Archives OF PATHOLOGY

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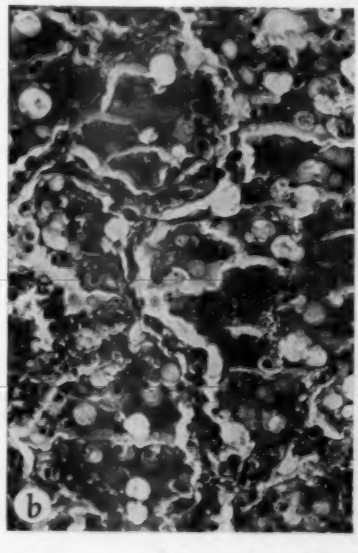

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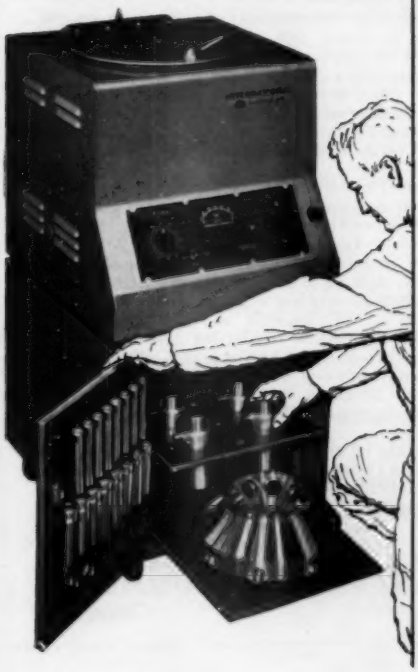

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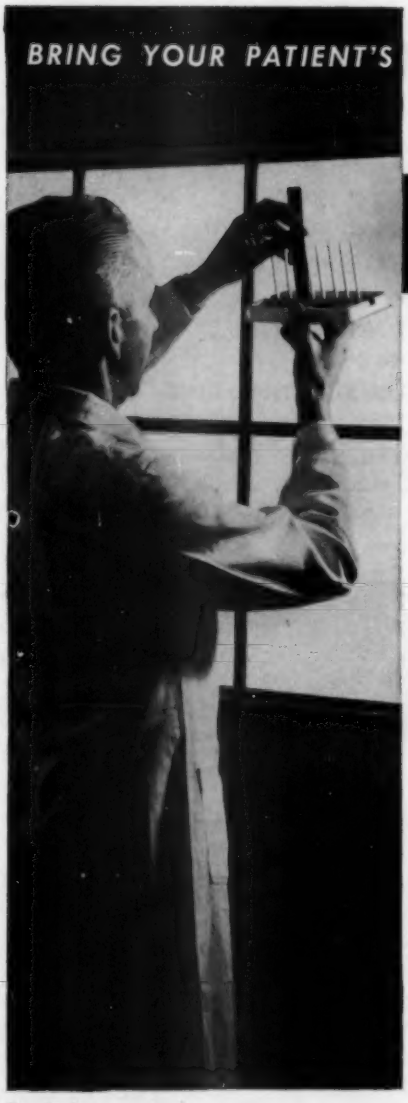

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Intimal Thickening in the Coronary Arteries in Infants

H. E. SCHORNAGEL, M.D.
Rotterdam, Netherlands

During life the structure of the coronary arteries is subject to continuous changes, caused by a variety of exogenous and endogenous factors, e. g., mechanical and hormonal factors and changes in the metabolism of the subject or of tissues of the arterial wall itself. In a large percentage of subjects these changes result in clinically manifest coronary sclerosis. In view of the interaction of so many different factors it is difficult, if not impossible, to retrace the history of coronary sclerosis found in persons of advanced age. A study of the structure of normal coronary arteries therefore seemed to be desirable.

As more or less marked pathological vascular changes, varying greatly in extent, are often found in middle-aged people, these subjects were unsuitable for study of the structure of normal coronary arteries. Investigations have, therefore, been made in subjects selected from younger and younger age groups, until finally neonati and infants up to the age of a year were chosen. A difference in structure between boys and girls in neonati was found by Dock.[2] Although his series was rather small and his technique not entirely satisfactory (he did not make serial sections), he believed that this difference, found at the beginning of life, is one of the causes of the predominance of coronary sclerosis in men as compared with women. This difference also was a starting point in the present investigation.

Submitted for publication June 23, 1956.
From the Pathological Laboratory of the Municipal Hospitals.

Methods

Hearts were used of 88 children—51 boys and 37 girls—varying from full-term stillborns to infants of a maximum age of one year. About 2 cm. of the ramus descendens anterior was removed and embedded perpendicularly in paraffin. Serial sections were made, and one of every 10 sections was mounted and stained alternately with hematoxylin-azophloxine and the Van Gieson elastin stain; this was sometimes supplemented by the Masson trichrome stain. If necessary, intermediate sections were studied. Frozen sections were examined in some cases in order to determine the possible presence of lipids. The changes found, e. g., splitting of the lamina elastica interna, and the development of the intima were graded and evaluated by figures ranging from 1 to 3 without the examiner knowing beforehand the age or sex of the subject in question.

Results

The results of this investigation may be summarized in Table 1, in which Grade 1 denotes that the endothelium rests on a lamina elastica interna of regular structure or that there exists only a splitting of the lamina elastica interna (Fig. 1), whereas Grade 3 denotes that a thick intima is present (Figs. 3 and 4), which change need not be circular (Fig. 2). Local factors are obvi-

ously of great importance; these are factors which may be correlated with the blood flow in the vicinity of ramifications (vibrations of the arterial wall).

Table 2 shows that increasing age, especially after the first month, is associated with increasing intimal thickening, which, in view of the constant percentage of nearly

TABLE 1.—*Intimal Thickening of Coronary Arteries in Infants*

Age Group	Sex	Number of Cases		
		Grade 1	Grade 2	Grade 3
<1 day	M	12	6	2
	F	16	3	2
1 day-1 mo.	M	5	2	1
	F	4	2	0
1-3 mo.	M	0	4	3
	F	0	2	2
3 mo.-1 yr.	M	2	3	11
	F	1	1	4

TABLE 2.—*Grade of Intimal Thickening in Coronary Arteries in Infants, Expressed in Percentages in Different Age Groups*

Age Group	Grades 2 and 3	
	Male	Female
<1 day	40.0%	23.8%
1 day-1 mo.	38.0%	34.0%
1 mo.-1 yr.	91.3%	87.5%

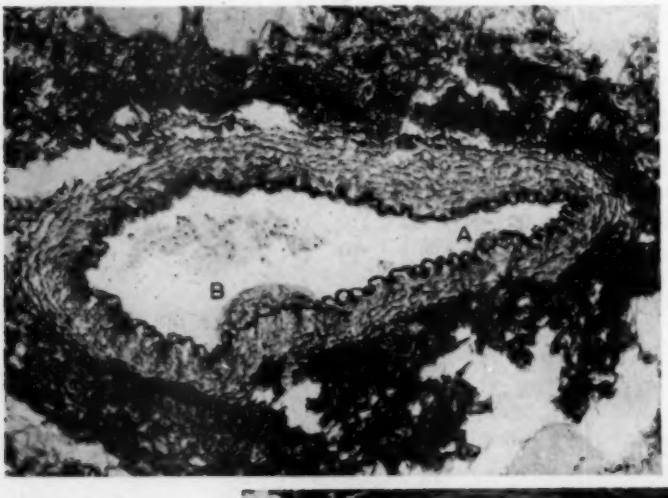

Fig. 1.—Coronary artery, female infant 21 hours old. At *A* intimal thickening Grade 1; at *B*, Grade 2 is suggested, but this point marks the beginning of a bifurcation. Elastin Van Gieson stain; reduced to 8/9 of mag. × 150.

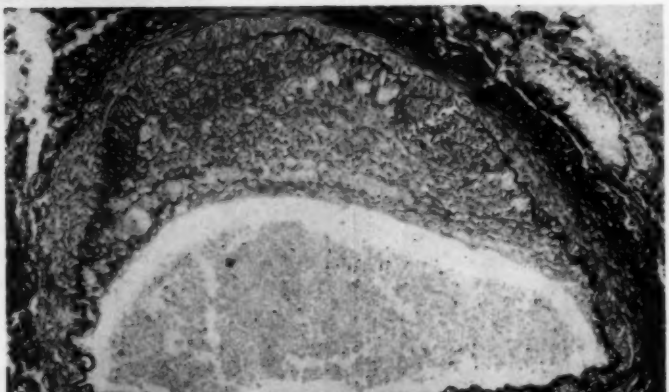

Fig. 2.—Coronary artery, male infant 20 minutes old. Intimal thickening Grade 3 with edema. Elastin Van Gieson stain; reduced to 8/9 of mag. × 100.

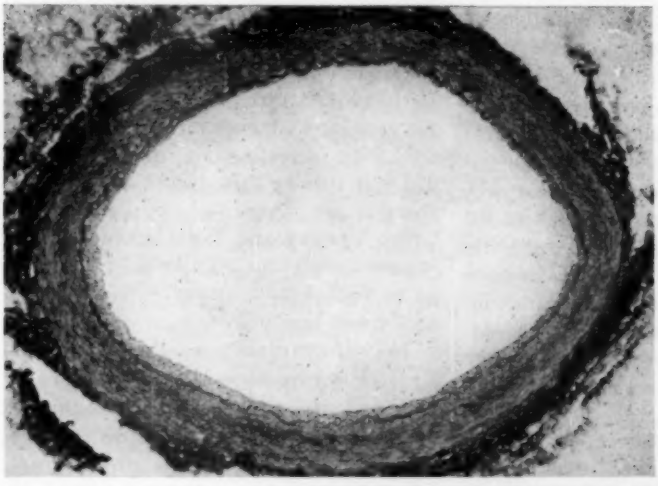

Fig. 3.—Coronary artery; male infant 12 months old. Intimal thickening Grade 3. Elastin Van Gieson stain; reduced to 8/9 of mag. × 60.

Fig. 4.—Coronary artery, male infant 9 months old. Intimal thickening Grade 3. Elastin Van Gieson stain; reduced to 8/9 of mag. × 70.

unchanged coronary arteries during the first month of life, requires some time to develop. It is demonstrated that boys show a slightly higher percentage of intimal thickening than girls.

Comment

This investigation, aimed at a study of the first changes in the structure of the vascular wall, was made in neonati and in infants, in whom the structure of the vascular wall is least complicated. The endothelium seems to rest directly on the lamina elastica interna, whereas the intima—where at a later age the most important changes, known as atherosclerosis, are localized—is not developed or hardly so. Transverse sections therefore often show no intima; according to Duff,[3] however, it is nevertheless often possible to recognize an intima if the arteries are studied from the endothelial side by a special technique.

The primary changes as a rule consist in splitting of the lamina elastica interna, followed by formation of the intima with the stellate cells of Langhans, i. e., cells of

a mesenchymal nature, the formation of elastic fibers, and ingrowth of muscle fibers. Detailed descriptions can be found in older reports by Wolkoff *; Gross, Epstein, and Kugel [8]; Ehrich, de la Chapelle, and Cohn [4]; Minkowski,[12] and others.

Dock [2] studied coronary arteries in neonati with a birth weight of 2500 to 3500 gm. who had died within 24 hours of birth. In several cases there was already some degree of change in the lamina elastica interna and some development of a subendothelial layer; the latter was found more frequently and showed more marked development in boys than in girls. Dock studied sections of each of the three large branches at a practically constant level. Short series of sections were made in some cases. In my opinion this does not suffice; the possibility should be taken into account that the section is not exactly transverse to the artery, so that distortion may exaggerate slight changes; it should also be borne in mind that the structure of the vascular wall in the vicinity of bifurcations may deviate greatly from the normal regular structure. These changes affect the intima in particular (Fig. 1, at *B*), but they are also often found in the media. Mention may be made in this respect of the so-called intimal cushions, described by Zinck,[19] Hirsch,[10] and Bucher [1] as occurring especially at vascular bifurcations. It is possible that these structures, which are normally present, have a function in the regulation of the blood flow (Hirsch [9]). If this is not taken into account, there is a grave risk of misinterpretation based on a single section of a coronary artery. In my investigations I have, therefore, used serial sections which, for practical reasons, were limited to only one coronary artery, i. e., to the ramus descendens of the left coronary artery. This branch was selected because many investigators believe that the majority of occlusions in old age are localized in this branch. Even if this is not generally accepted, the fact remains that the first and the most marked sclerotic changes occur in the ramus descendens anterior.

The results of this investigation show a fairly marked variation in the structure of the vascular wall, even in infants. Progression with increasing age is unmistakable, and it is striking that marked, if only local, changes are frequently seen in infants. Gross, Epstein, and Kugel [8] point out that in some cases the fibroelastic intima may be as thick, locally, as the media at the end of the first year of life.

The difference between the sexes found by Dock in neonati was confirmed by Fangman and Hellwig,[5] whereas Lober [11] and Minkowski [12] believed they had demonstrated this difference only after the first month of life. I have been unable to confirm Dock's findings fully; although some difference between the sexes was found in the neonati, it was not significant.

The question arises as to which factors are of importance in the pathogenesis of these changes and whether these changes are of any importance in the pathogenesis of coronary sclerosis.

As regards the pathogenesis of intimal thickening, i. e., of the formation of an elastic, hyperplastic intima, it is generally believed that this represents a phenomenon of adaptation to the high demands already made on the coronary arteries during the first months of life, continuing in varying degree in later life. The coronary arteries are in a relatively unfavorable position as compared with the other arteries. As a branch of the first part of the aorta, they are (intermittently) pumped full with blood; immediately afterward, during the systole, the drainage of blood is markedly inhibited by contraction of the myocardium; this results in congestion associated with an increase in the lateral pressure to which the vascular wall is subject. They have, moreover, a fixed starting point in the aorta and, subsequent to their subepicardial course, a point of fixation at which they penetrate the muscular wall. During the cardiac action these two points have a slightly varying

* References 15-17.

distance (Dock), as a result of which the coronary arteries, so to speak, are alternately elongated and shortened. These two factors increase the demands made on the arterial wall. In addition, the subepicardial course of these arteries precludes outside support. The importance of this anatomic fact was pointed out by Geiringer,[7] who found that, if a muscular bundle passes over the coronary artery (the part which he refers to as mural coronary artery, in contrast with the normal epicardial coronary artery), vascular changes in the mural part are considerably less severe than in the epicardial part. The wall of the epicardial coronary artery may be thicker by 33% than that of the mural part. This is a result of intimal hyperplasia; Geiringer[7] agrees with other investigators, therefore, that an increase in lateral pressure gives rise to intimal hyperplasia and that overstretching would seem to be the most important stimulus to intimal growth. This intimal thickening should be interpreted as a normal physiological phenomenon of adaptation, which is more marked in boys, with their slower cardiac action and larger stroke volume and therefore greater demands on the coronary arteries, than in girls. The broader intima gives rise to other conditions in the vascular wall itself, e. g., the conditions ensuring a satisfactory metabolic state of the vascular wall. It is therefore not surprising that fatty infiltration is sometimes seen in this thick intima, even in infants, as I was able to demonstrate in some a few months of age. This fatty infiltration is thought to be reversible and can be found especially in association with infectious diseases. Nevertheless, this accumulation of fat suggests metabolic disturbances. Geiringer,[7] too, pointed to this when he emphasized that thickening of the intima interferes with the normal metabolic condition of the vascular wall unless special changes occur in the vascular structure. Normal nutrition, which takes place by diffusion from the lumen, becomes insufficient when the intima shows a critical thickness of 0.35 mm. in the descending branch of the left coronary artery (Geiringer[6]). If the intima becomes thicker, vascularization of the vascular wall itself must occur if the nutrition of the wall is to be kept at a normal level. In the case of gradual intimal thickening, this vascularization takes place by ingrowth of capillaries from the media to the intima as a result of the stimulus of the disturbed metabolic equilibrium (insufficient oxygen tension). In the case of rapid intimal thickening resulting from the organization of larger thrombi vascularization takes place from the lumen. This is found in the severe changes at advanced age for which the basis comes into existence shortly after birth.

It is often difficult to distinguish between physiological and pathological processes; in this respect, Lober[11] states that "there is no point at which the artery can be said to have stopped growing or developing and started to degenerate or become diseased." This has been confirmed by other investigators, e. g., by Moschcowitz,[13] who states that "the evolution of hyperplastic arteriosclerosis begins at birth as an irreversible process of adaptation." Wilens[14] goes so far as to suggest that "the diffuse overgrowth of the intimal tissue that occurs consistently early in life is not a natural developmental phenomenon but is related to the same mechanism that eventually leads to the formation of atherosclerotic plaques." This observation is based on his postulation that this intimal thickening is never encountered in animals which show no spontaneous arteriosclerosis. If this is the case, the theory should be accepted that man possesses a vascular apparatus which is congenitally inferior for his existence and which guarantees only a limited life span. This theory is occasionally expressed in the saying "a man is as old as his arteries." However, investigations by Wolkoff,[16] who compared several animal species, did, in fact, reveal some intimal development, although invariably less marked and simpler in nature than in man. The intimal changes mentioned must principally be regarded as

the result of hereditary, as well as mechanical, factors. Although I am inclined to regard this process as a physiological phenomenon of adaptation, I am convinced, on the other hand, that this intimal thickening provides a basis for the coronary sclerosis regularly encountered in old age.

Summary

A study was made of the coronary arteries of infants up to the age of a year. Special attention was given to the gradual thickening of the intima and its significance in the pathogenesis of coronary sclerosis. Intimal development is often obvious in infants after the first month of life and may be slightly more marked in boys than in girls.

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A Study of Toxic Doses of Strontium-90 (Sr^{90}) in the Adult Rat

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Introduction

An outstanding metabolic characteristic of radioactive strontium is its selective deposition in the skeleton. Three days after injection the total accumulation in soft tissues represents less than 1% of that in bone.[1] Moreover, it is eliminated very slowly from the skeleton. According to a report from the Argonne Laboratories by Anthony and associates,[1] when strontium-89 (Sr^{89})* is injected intraperitoneally into adult rats, they retain about 59% of the injected dose. Anthony, Lathrop, and Synder[2] reported that when Sr^{89} is given as a single intraperitoneal injection to adult rats, the dose which produces 50% mortality in 30 days (L. $D._{50}/30$) is slightly less than 5μc per gram of body weight. Other investigators have studied the radiotoxicity and pathologic effects of Sr^{89}. For example, it is known that Sr^{89} produces bone tumors in mice,† rats,‡ rabbits,§ and dogs.‖

The toxic effects of Sr^{90} are less well known. It is just recently that reports have been published on the toxicity and pathologic effects of Sr^{90}. Finkel and Scribner[12] induced bone tumors in mice by single intravenous injection of Sr^{90}. Some data are available on the toxic effects of Sr^{90} in other species.¶ The L. $D._{50}/30$ days for Sr^{90} is reported to be 6μc per gram for the mouse[12] and 0.15 mc. per kilogram for dogs.[11] A single injection of 0.2 mc. per kilogram is fatal to monkeys.[16] To our knowledge, the only published report on the effects of Sr^{90} in rats is one by Ray and associates.[17] Therefore, it seemed advisable to study further the effects of Sr^{90} in rats.

Materials and Methods

Rats.—Virgin females of the Sprague-Dawley strain were obtained from Sprague-Dawley, Inc., in Madison, Wis. The initial injections of Sr^{90} were given when the rats were 3½ months old.

Strontium-90 (Sr^{90}).—One shipment of radioactive strontium from the Oak Ridge National Laboratory sufficed for all of the injections. This shipment contained the carrier-free chlorides of Sr^{90} and Sr^{89} in a weak solution of hydrochloric acid. Subsequent dilutions were made with distilled water.

At the beginning of the experiment, the injected solutions contained 95.5% Sr^{90} and 4.5% Sr^{89}. The proportion of Sr^{90} to Sr^{89} decreased thereafter because of the difference in half-lives. Sr^{89} decays with a half-life of 53 days and a maximum beta

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* Much of the initial work with radioactive strontium at the Argonne Laboratories was done with fresh preparations of fission strontium, which contained a high percentage of Sr^{89} and smaller amounts of Sr^{90}. Therefore, the radiation effects shortly after injection were due almost entirely to SR^{89}. In their experiments, it was only after several half-lives of Sr^{89} (53 days) that Sr^{90} represented a significant percentage of the total retained radioactivity.

† References 3-8.
‡ References 3, 4, 9.
§ References 3, 4.
‖ References 10, 11.
¶ References 11, 13-17.

energy of 1.463 mev to stable yttrium. Sr^{90}, with a half-life of 25 years and a maximum beta energy of 0.61 mev, decays to yttrium-90 (y^{90}). Y^{90} has a half-life of 2.54 days and a maximum beta energy of 2.18 mev. It decays to stable zirconium.

Sr^{90} and Y^{90} were in radioactive equilibrium # when injected, at which time there were about 3600 atoms of Sr^{90} present for each atom of Y^{90}.

Plan of Experiments.—The 120 rats were divided into six groups of 20 each, and single injections were given intraperitoneally in doses ranging from 0.1 to 5 μc of Sr^{90} per gram of body weight (Experiment A, Table 1).

TABLE 1.—*Plan of Experiment A on Sprague-Dawley Rats with Sr^{90}*

No. of Rats	Single Intraperitoneal Injection of Sr^{90}, μc/Gm. Body Weight
20	5.0
20	3.5
20	2.5
20	1.0
20	0.5
20	0.1

TABLE 2.—*Plan of Experiment B on Sprague-Dawley Rats with Sr^{90}*

No. of Rats	Monthly Intraperitoneal Injection of Sr^{90}, μc/Gm. Body Weight	Total Dose of Sr^{90} in 5 Monthly Injections, μc/Gm. Body Weight
20	1.0	5.0
20	0.7	3.5
20	0.5	2.5
20	0.2	1.0
20	0.1	0.5
20	0.02	0.1
20 (untreated controls)	0	0

The same number of rats were used in a monthly injection series (Experiment B, Table 2), and it was intended that they should receive in five monthly injections the same total dose of Sr^{90} per gram as those rats given a single injection; however, the planned total dose was not administered in some groups, i. e., when death occurred before the five monthly injections were completed. Twenty rats of the same shipment were maintained as controls, as well as fifty additional rats of the same strain but from other shipments.

It was planned that 10 rats of each experimental group of 20 (Experiments A and B) were to be used to study survival and late-effects of Sr^{90}, while 2 rats of each group were to be killed at each of the following times after the initial injection: one week and one, three, five, and seven months. This schedule was followed without deviation in those groups in which survival was good. Histologic examination was made of the viscera and femur of all animals.

The experiment was terminated nine months after the initial injections, at which time all of the remaining rats were killed. Histologic sections were prepared of two rats in each of these surviving groups.

The rats were weighed at the beginning of the experiment,* at the time of monthly injections of Sr^{90} (Experiment B only), and when they were killed. Beginning six months after the initial injection of Sr^{90}, the surviving rats were weighed and x-rayed once a month in order to detect development of bone lesions.

Results

Mortality.—As previously mentioned, 10 rats of each experimental group of 20 were used to study the mortality rate and survival with each dose (Tables 3 and 4). The data on mortality are presented graphically in Figures 1 and 2. All rats given single injections of 5.0, 3.5, and 2.5μc of Sr^{90} per gram were dead in two months. In four months the 1.0μc-per-gram group was also dead. A single dose of 2.5μc per gram produced 60% mortality in 30 days. Ray and associates [17] estimated from their data that the L. $D._{50}$/50 days for Sr^{90} in young Long-Evans rats (31-44 days) was 2.5 to 3.0μc per gram of body weight. The L. $D._{50}$/30 days for Sr^{89} in rats is reported to be slightly less than 5.0μc per gram.[2]

Although it appears from Figures 1 and 2 that the survival rate was much better in the divided-dose (monthly-injection) groups, this is actually not so. The time of death

When a parent element decays, the relative quantities of parents and daughters eventually reach an equilibrium so that the number of atoms of parent isotope to that of the daughter isotope is in ratio with their half-lives.[18] In the present experiment, the stock solution of radioactive strontium from Oak Ridge was allowed to stand over two months before it was used.

* At this time the experimental and control rats were approximately the same weight, i. e., about 235 gm.

TABLE 3.—*Experiment A: Mortality of Rats Given Single Intraperitoneal Injection of Strontium⁹⁰*

Dose of Sr^{90}, μc/Gm. Body Weight	No. of Rats Used to Study Mortality Rate	No. Dead, Days After Injection					Average Time of Death, Days After Injection*
		15	30	45	60	270, i.e., just Before Termination of Experiment	
5.0	10	10	10	10	10	10	12
3.5	10	7	8	9	10	10	20
2.5	10	4	6	7	10	10	28
1.0	10	0	0	1	1	10	99
0.5	10	0	0	0	0	9	194
0.1	10	0	0	0	0	2	232

* Exclusive of those rats which were killed at the termination of the experiment.

after the last injection is closely related to the total dose administered, and there is considerably less difference if one takes the date of death in respect to the total dose, whether it be in the single- or the monthly-injection group. For example, in Table 4, Experiment B, the animals receiving 1.0μc per gram per month died on the average of nine days after the third dose (total of 3.0μc per gram), while a single injection of 3.5μc per gram caused death on the average in 20 days. Likewise, a total of 2.1μc per gram in three monthly injections produced death on the average in 19 days after the final injection, whereas 2.5μc per gram in a single injection caused death on the average in 28 days. The radiation effects of previously injected doses shortened the life span after the final injection, so that survival is actually shortened in the divided-dose ainmals if one considers the total dose and the time of death after the administration of the last fraction of the dose.

Weight Gain.—In Experiment A (Fig. 3), single injections of Sr^{90}, the animals receiving 5.0 or 3.5μc per gram showed an immediate failure of weight gain and actual loss of weight. The other four groups, that is, 2.5, 1.0, 0.5, and 0.1μc per gram, showed a temporary weight gain within the first week after injection, and then a continuous decline of weight, so that in approximately three weeks the control base line was reached. Thereafter, all groups given single injections of Sr^{90} showed a depressed weight gain. The failure of weight gain at

TABLE 4.—*Experiment B: Mortality of Rats Given Monthly Intraperitoneal Injections of Sr^{90}*

Monthly Dose of Strontium ⁹⁰ μc/Gm. Body Weight	No. of Rats Used to Study Mortality Rate	No. Dead, Days After Initial Injection				Av. Time of Death, Days After Initial Injection*	Av. No. of Monthly Injections Given Before Death	Av. Total Dose, μc/Gm. Body Weight
		60	90	180	270, i.e., Just Before Termination of Experiment			
1.0	10	0	10	10	10	60	3	3.0
0.7	10	0	9	10	10	79	3	2.1
0.5	10	0	1	10	10	119	4	2.0
0.2	10	0	0	4	7	194	5	1.0
0.1	10	0	0	3	6	191	5	0.5
0.02	10	0	0	0	5	251	5	0.10
Untreated controls	10	0	0	0	3	235	0	0

* Exclusive of those rats which were killed at the termination of the experiment.

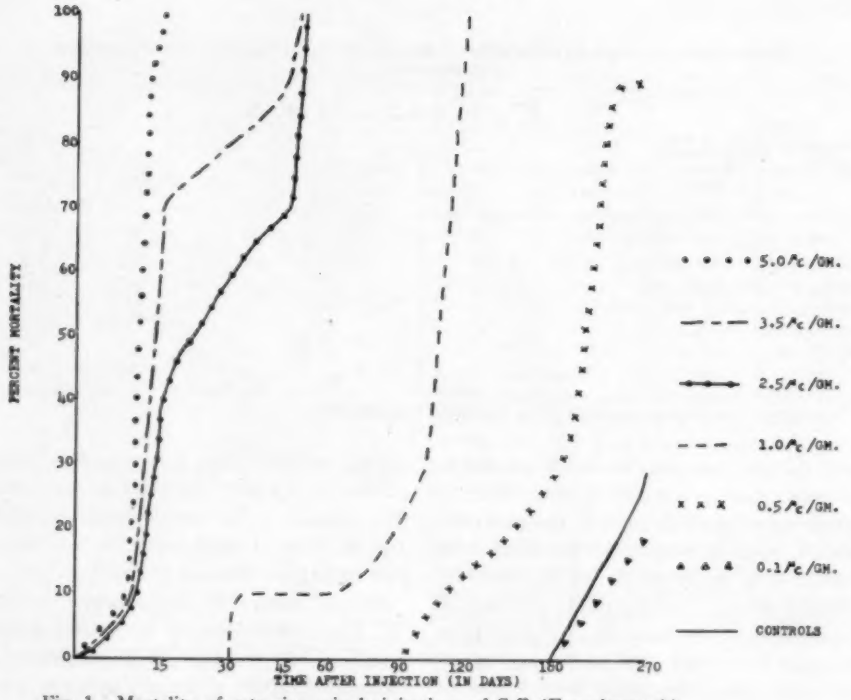

Fig. 1.—Mortality of rats given single injections of Sr^{90} (Experiment A).

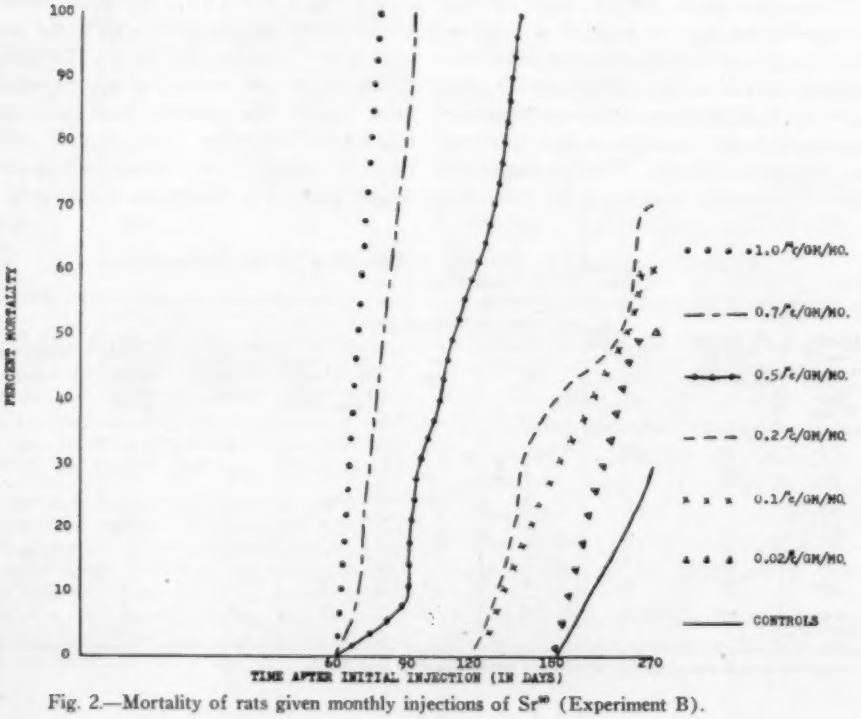

Fig. 2.—Mortality of rats given monthly injections of Sr^{90} (Experiment B).

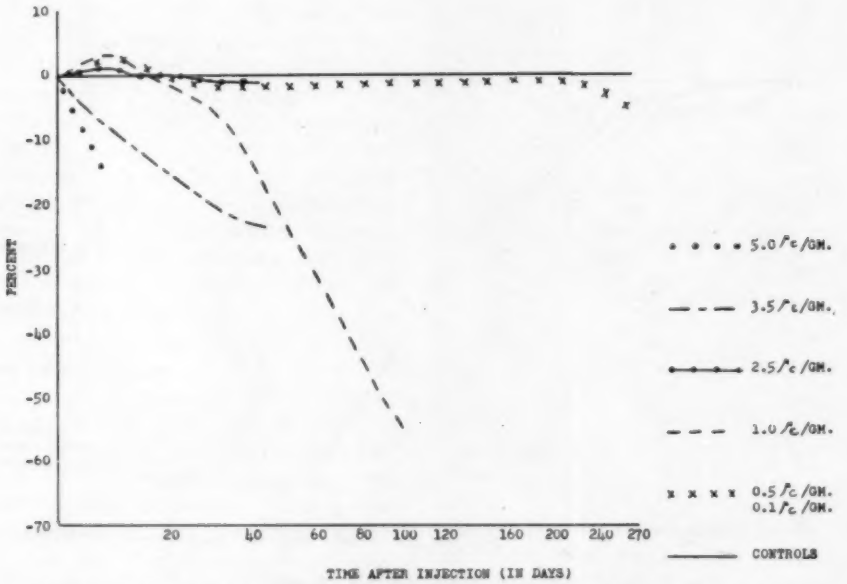

Fig. 3.—Differences between the percentage gain in weight of the controls and the rats given single injections of Sr^{90}. (Experiment A).

any time was most marked in the group surviving with the largest dose of Sr^{90}. For example, at 60 days after injection, no rats injected with more than 1.0μc per gram survived, and, among the surviving groups, those rats injected with 1.0μc per gram showed the most drastic loss in weight.

In Experiment B (Fig. 4†), monthly injections of Sr^{90}, those rats given 0.2μc per gram per month gained slightly more weight than the controls within the 1st week to 10 days but fell below the control base line in the 3d week.

The other groups showed a depressed weight gain. After the third week those rats injected with 0.7 and 0.5μc per gram per month showed a decided loss in weight.

In some groups the gain in weight during the first week following injection (Figs. 3 and 4) was slightly more than that of the controls but was not significant, and may be attributed to biological variations rather than to an effect of Sr^{90}.

Pathologic Effects.—Between one and two weeks after injection the rats receiving a single dose of 2.5 to 5.0μc of Sr^{90} per gram developed a bloody crust around the nose and eyes. This may have been a manifestation of radiostrontium toxicity and, as Ray and associates[17] suggest, the development of bloody noses in rats following injection of Sr^{90} indicates increased tendency toward hemorrhage or upper respiratory tract infection. Similar observations have been reported following injection of toxic doses of Sr^{89}.[2]

The weight gain and growth were depressed in the higher dose groups, as shown in the Tables. The decrease in growth is also recorded radiographically. Only one animal in this series suffered a fracture which involved the right tibia. This is contrary to the experience of Bloom,[19] who reported frequent fractures following injection of Sr^{89} in rats, which, however, were much younger than ours at the time of injection.

† The three lowest dose groups, i. e., 0.2, 0.1, and 0.02 μc/gm/mo., showed the same general trend in weight. For the sake of clarity, they were averaged and one curve plotted in Figure 4.

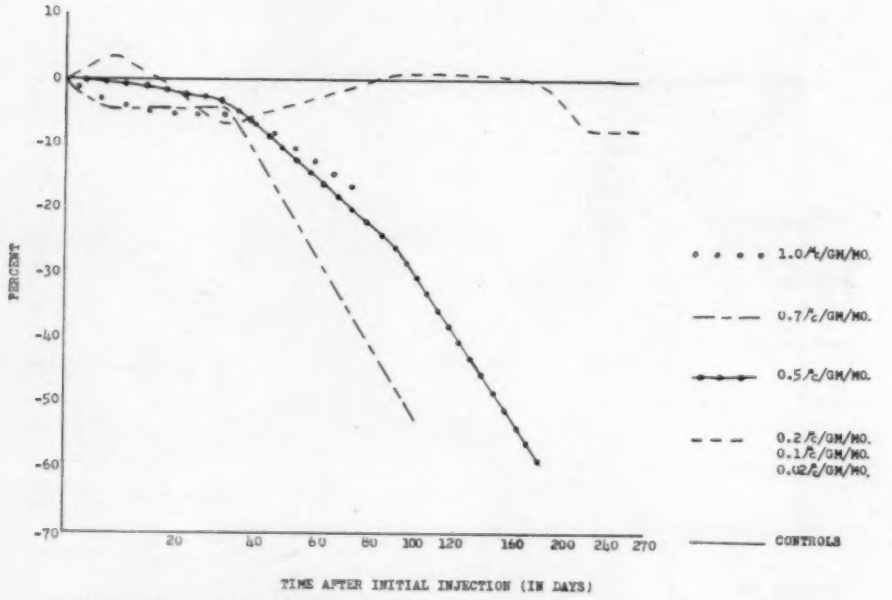

Fig. 4.—Differences between the percentage gain in weight of the controls and the rats given monthly injections of Sr^{89} (Experiment B).

Microscopic Changes.—The early, and the only consistent, pathologic effects were found in the bone marrow. In the single-injection animals this was quite obvious in the highest-dose group. One week after injection of 5.0μc per gram of body weight the femur marrow showed loss of all hematopoietic activity and was almost entirely composed of hemorrhagic fat tissue. One week after injection of 3.5 and 2.5μc per gram the effect was quite similar except that in the center of the shaft of the femur some marrow remained and showed evidence of slight hematopoietic activity. Doses of 1.0 and 0.5μc per gram produced a marked degree of marrow hypoplasia in the metaphysis, but active hematopoiesis was present in the central part of the shaft. The marrow aplasia continued, and death was in large measure caused by the hematopoietic disturbance. Comparatively little effect was noted in the metaphyseal ends of the femur one week after injection of 0.1μc per gram, although decrease of marrow cellularity was noted in some. In three months the animals given single injections of 0.5 and 0.1μc per gram still showed some fatty, acellular marrow in the metaphysis but rather active hematopoiesis in the central portion of the shaft. From that point on there is gradual recovery of hematopoiesis.

All histologic changes are related to the total dose administered, and the cumulative dose of interrupted injections produces effects quite similar to those of a single injection. Obviously, however, the effects of interrupted injections of a given total dose do not develop as rapidly as after the same dose given in a single injection.

The effect on the bone itself was one of injury to the growing trabecular bone immediately adjacent to the epiphyseal cartilage plate. The effects are quite similar to those observed with Sr^{89}, as so well documented by Bloom.[19] The epiphyseal plate becomes wider; the cartilage cells on the metaphyseal side become quite large and ballooned, and the columns of cartilage cells are irregular. The bony trabeculae show loss of osteocytes and osteoblasts within the first week in the high-dose group.

Gradually there is some distortion of the metaphyseal trabeculae, a number of them becoming wider than usual and some of them separated from the epiphyseal plate. This has been referred to as severance, but in the age group of animals used in this experiment it is not nearly as prominent as it is in younger animals, as recorded by Bloom.[19] After several months there is evidence of considerable bony thickening from persistence of cartilage within the trabeculae, and of irregular ossification. In this metaphyseal zone there is eventually marked widening of the bony trabeculae, which may be incorporated in actively growing fibrous tissue of the marrow spaces. In three of the animals a distinct central mass of irregular fibrous tissue was formed in the metaphysis. There is also some irregularity of endosteal bone formation and the development of irregular, poorly calcified foci of endosteal bone. Eventually, in three of the animals the proliferating fibrous bone showed foci of considerable cellular activity and a pattern which has histologic criteria of malignant bone tumor.

Comment

Sr^{90} is a bone seeker and accumulates in the most actively growing ends of bones and produces severe damage in the marrow. The site of deposition can be observed in Figure 5, which is an autoradiogram of a femur of a rat killed one week after injection of 0.02μc of Sr^{90} per gram of body weight. The heavy blackening on the endosteal side and on the shaft side of the epiphyseal plate shows the concentration of radioactive strontium at these sites.

Depletion of the hematopoietic activity in bone marrow following injection of Sr^{90} is similar to that of Sr^{89}. The maximum change is related to the site of greatest skeletal deposition of Sr^{90}. The strontium is deposited in the mineral structures of the actively growing bone, and the small marrow spaces in the ends of the bone are subjected to greatest bombardment of beta particles. Beta particles travel only a few millimeters in tissue and therefore do not have much effect on the marrow in the central part of the shaft, where strontium-burdened trabeculae are not present.

Fig. 5.—Femur autoradiogram of a rat killed one week after an intraperitoneal injection of 0.02 μc of Sr^{90} per gram of body weight. Ansco No-Screen Film.

It is noted that a single injection of 2.5μc of Sr^{90} per gram produces a mortality of 60% in 30 days, whereas 2.5μc of Sr^{89} in 10 consecutive daily doses produces a similar mortality in 60 to 90 days.[9] This is in agreement with Finkel,[12] whose studies have shown that beta-emitters with relatively long half-lives are twice as lethal during the acute period as those with relatively short half-lives, and that there is a linear relationship between energy and lethality. Therefore, during the acute period following injection, Sr^{90} is more lethal than Sr^{89}.

In this experiment only a few of the animals were allowed to survive to the time when tumors were beginning to develop. However, Sr^{90} does produce bone tumors and perhaps is more effective than Sr^{89}.

Conclusions

1. Strontium-90 (Sr^{90}) is a bone seeker and is chiefly incorporated into the mineral architecture of actively growing bone. It causes severe hematopoietic suppression and radiation injury of growing bone, which is followed by repair and, in time, by osteogenic sarcoma.

2. Sr^{90} is more potent than Sr^{89} in all of its radiation effects. This greater effect is related to its long half-life and its radioactive daughter Y^{90}, which has a high maximum energy and with which it is in equilibrium.

3. The L. $D._{50}$/30 days for Sr^{90} in the rat is slightly less than 2.5μc per gram.

4. Multiple administrations of Sr^{90} are cumulative, and the biological effects are a function of the total dose.

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Studies of Interstitial Radiolutetium (Lu^{177}) in the Dog

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Since radioactive isotopes have become available for medical usage, their usefulness as tools of investigation has certainly surpassed their utility as therapeutic instruments. The ease with which they can be combined with chemicals, traced, and located with precision within the body makes them adaptable for a wide range of tracer studies. Therapeutic usage has reached the stage of practicality with Co^{60} as a source of high-intensity irradiation. Various techniques of brachytherapy have been developed in which the isotope is incorporated into needles, spheres, tubes, and so forth, and placed into, or in approximation with, neoplasms in one manner or another.

A more physiologic adaptation of isotopes as a source of selective irradiation incorporates the radioactivity in a metabolic manner into the tissue which one is desirous of irradiating. The most successful metabolic usage to date has been with I^{131} in treating hyperplasia of the thyroid gland. The usefulness of this isotope in treating carcinoma of the thyroid has been limited by the lack of function exhibited by the vast majority of thyroid cancers.

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A theoretical quasimetabolic adaptation that has promoted considerable attention is based on the avidity that lymph nodes have for colloids of varying particle sizes. Radioactive colloidal gold 198 (Au^{198}), colloidal chromic phosphate containing phosphorus 32 (P^{32}), and yttrium 90 (Y^{90}) in ionic preparations containing stable yttrium carrier and showing colloidal behavior in tissue have all been studied.

Lutetium 177 (Lu^{177}) is another radioisotope which might have some advantages as a source of selective radiation for the reticuloendothelial system. Like other rare earths, hydrolysis of ionic lutetium approaches completion before reaching the pH of blood. The hydroxide is insoluble, and its rapid removal from the blood and deposition largely in the liver and spleen suggest colloidal properties.*

By reaction with neutrons the naturally occurring mixture of Lu^{175} and Lu^{176} yields Lu^{176} and Lu^{177}, respectively. The half-lives of the artificially produced isotopes are 3.7 hours and 6.8 days, respectively.

Lu^{177} decays in part to stable hafnium 177 (Hf^{177}) by emission of 495-kev beta rays and in part to two levels of excited Hf^{177} by emission of beta rays of lower energies. The spectrum of excited Hf^{177} includes gamma rays with energies of 318, 206, and 112 kev. The lowest-energy beta group yields an excited state followed by a cascade of 206- and 112-kev gamma rays or by a crossover of 318-kev gamma rays to the extent of 4.5%.[2] The intermediate-energy beta group yields an excited state 112 kev above that of stable hafnium and

* Kyker, G. C.: Studies of Lu^{177}, Ho^{166}, Y^{90}, and Y^{91} in Animals: Unpublished data.

decays to the ground state by gamma omission.

Lutetium is primarily a beta-particle emitter. The average energy per disintegration of these particles is 0.12 mev/day, which is relatively low. Most of the radiation energy is absorbed by the tissue very close to the point of deposition of Lu^{177}. One-half of its beta absorption will be within a radius of 0.28 mm.† This relatively short penetration can be advantageous only if uniform chemical distribution of the isotope can be achieved. The gamma-ray emission probably aids only slightly in smoothing out the effect of uneven chemical distribution.

The following animal experiments were designed to establish whether or not radiolutetium behaves as a colloid when injected into the mammary gland of a dog. It was further desired to study the degree and rapidity of lymph-node concentration, the intranodal distribution patterns of the isotope, the general dosage required to produce lymph-node changes, the general body effect, and the tolerance of the tissues at the injection site.

Lutetium oxide was obtained from Dr. F. H. Spedding, Director, Ames Laboratory, U. S. Atomic Energy Commission, Ames, Iowa. It was activated for three weeks in the Oak Ridge graphite reactor and radioassayed by an absolute beta-counting procedure that was verified by NBS standards. Two groups of female dogs were used. Eleven animals received injections of 1 to 2 mc. in the right superior mammary gland. The animals were then autopsied at varying intervals, ranging from two hours to three weeks. Assay material was obtained from the superior mammary gland, heart, lungs, kidneys, liver, spleen, sternum, ribs, pectoral muscles, thyroid gland, urinary bladder, gall bladder, pancreas, omentum, and lymph nodes of the abdomen, chest, axilla, and neck of each dog.

The specimens were prepared for radioassay by wet ashing in hot nitric acid. The beta-counting rates of suitable aliquots were measured with a thin end-window Geiger-Müller tube.

One-half of each lymph node was retained for histological examination and autoradiograms. Two or three sections were mounted on N. T. B. plates and developed at varying lengths of time. The shorter exposures served to demonstrate the distribution of the isotope. The longer exposures showed the extent of radiation effect.

A second group, of eight dogs, received intramammary injections of larger doses, ranging from 24 to 131 mc. of Lu^{177}. These animals were autopsied four to five weeks later. Tissues were obtained from the mammary glands; liver; spleen; bone marrow; kidneys, and the axillary, internal mammary, latissimus dorsi, cervical, and mediastinal lymph nodes. The lymph nodes from the left side of the body served as controls. Both histological sections and autoradiograms were prepared.

Results

Relatively high concentrations of radioactivity were found in the right axillary, right internal mammary, and right latissimus dorsi nodes. The cervical lymph nodes rarely had appreciable activity. The control nodes from the left half of the body had very little activity. Significant counts were never found in the other organs, such as marrow, kidneys, liver, or spleen.

Very little radioactivity was demonstrated in the regional lymph nodes in two or three hours. Within two days, however, there was considerable concentration of the isotope within these nodes, as demonstrated by both cytological radiation changes and by autoradiograms. It was thus demonstrated that the isotope did concentrate in the nodes and did so well within the half-life of 6.7 days.

The animals receiving the higher curiage were examined with the thought of determining the tolerance of the local site of injection to the isotope, the extent of the radiation changes within the node, and the intranodal distribution of the isotope.

Lu^{177} was well tolerated at the injection site. Even with 131 mc. radiation necrosis did not occur. The tissues of the breast and skin were somewhat edematous and showed both inflammatory and radiation changes, but the degree of change was not comparable to the changes encountered when Au^{198} is injected into the mammary gland of the dog. Radiation changes in the

†Kyker, G. C.; Christopherson, W. M.; Berg, H. F., and Brucer, M.: Selective Irradiation of Lymph Nodes by Radiolutetium (Lu^{177}): Unpublished data.

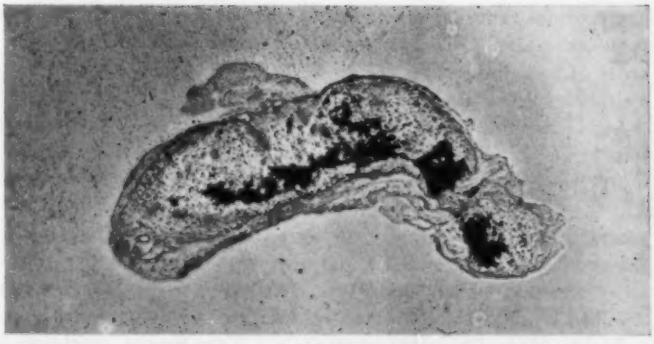

Fig. 1. — Autoradiogram of right intercostal lymph node five weeks after injection of 43 mc. of Lu^{177} into right superior mammary gland. Note the diffuse irradiation effect and total destruction of the node.

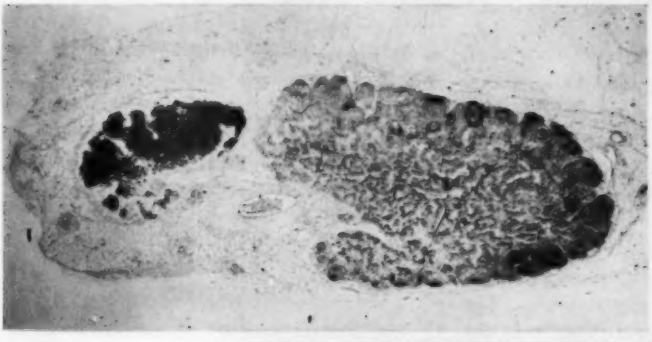

Fig. 2.—Autoradiogram of right axillary lymph node six weeks after intramammary injection of 131 mc. of Lu^{177}. The radioactivity and destruction are confined to only a portion of the node.

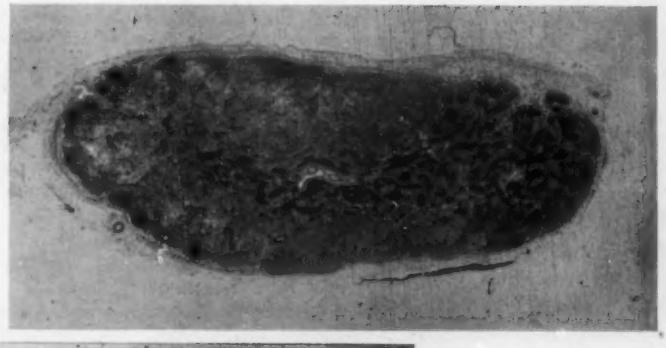

Fig. 3. — Hematoxylin-eosin section of right axillary lymph node six weeks after injection of 43 mc. of Lu^{177}. Destruction is patchy and incomplete.

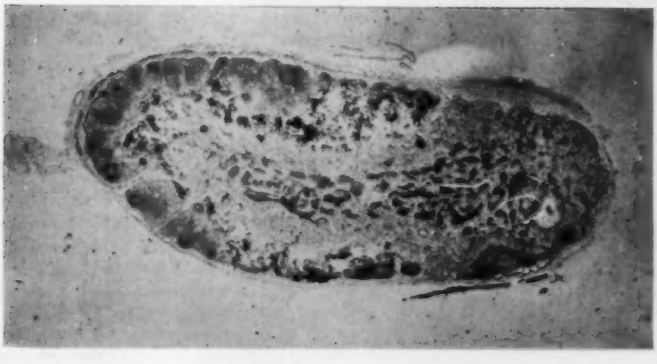

Fig. 4.—Autoradiogram shows distribution of activity corresponding to necrosis pictured in Figure 3.

lymph nodes ranged from complete necrosis in some nodes (Fig. 1) through patchy areas of radiation change (Fig. 2) to completely unaltered nodes. Qualitatively, the changes encountered were similar to those found in irradiation from an external source or from interstitial injection of Au^{198}. Quantitatively the changes were somewhat less impressive than those produced by radiogold, in that fewer lymph nodes were completely destroyed. The isotope had no demonstrable effect on the general state of health of the animals.

Comment

These experiments support the data that lutetium behaves as a colloid at body pH. This property would place the isotope in the realm of those theoretically useful for irradiating the reticuloendothelial system of the body in a more or less selective manner. Perhaps some conclusions as to the limitations of its usefulness in this regard can be drawn from these experiments. The great limiting factor, as with Au^{198}, is the nonhomogeneous intranodal distribution of the isotope.[1] In the case of Au^{198} this lack of uniformity in distribution is somewhat negated by the relatively stronger gamma component. Lutetium has fewer gamma radiations per disintegration, and they are of lower energies. Although Lu^{177} appears to be inferior to Au^{198} in this respect, it has other characteristics which are in its favor. The half-life of 6.7 days, as compared with 2.7 days for Au^{198}, would make it more useful when distance of shipping is a factor. In this respect the low-energy gamma radiations make it easier to ship and less hazardous to handle. Finally, the tissues tolerate the local concentration of lutetium much better than they do Au^{198}. Severe reactions at the injection site are thus avoided.

Whereas the shortcomings of this potential tool have been emphasized, these apparent defects do not necessarily present an insurmountable problem. More study is needed to determine whether or not a better uniformity of intranodal distribution of the isotope can be obtained. Such studies are now in progress.

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Carcinoma of the Liver in Rats Fed Ethionine

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In 1953, Popper and co-workers[1] reported that rats fed for about 100 days on a diet containing ethionine developed tumor-like nodules in their livers. Certain histologic features suggestive of malignancy were present, but evidence of biological malignancy, such as invasion, metastasis, or transplantability, were not presented in this or in a subsequent confirmatory report.[2] In an effort to determine whether an unequivocally malignant neoplasm would be induced by ethionine, animals were maintained for longer periods of time on a semisynthetic diet containing ethionine. Malignant tumors showing invasion and metastases were observed. The characteristics of these neoplasms are the subject of this report.

Experimental Study

White Wistar rats (Carworth Farms) of both sexes, weighing from 150 to 200 gm., were used. In the first experiment, 30 animals were divided into three groups of 10 each, 5 of each sex. The control group was fed a basal 16% casein diet[a]; a second group, the basal ration containing 0.25% DL-ethionine (California Research Foundation) in place of a corresponding amount of sucrose, and the third group, the same ration with 0.5% DL-ethionine. The animals, five to a cage, were housed in screen-bottomed cages in an air-conditioned room and were weighed twice weekly. One male and one female of each group were killed at 60, 90, and 120 days after beginning the experiment. The pathologic findings in these animals were reported previously.[3] Four animals on the 0.25% ethionine diet and four on the basal diet without ethionine died or were killed between eight and a half and nine months after the initiation of the diet. All the animals on the 0.5% ethionine diet died within 120 days. In a second experiment, 10 animals, 5 of each sex, were killed at the end of 10 months, after being fed the basal diet containing 0.25% ethionine for 8 months, followed by 2 months of stock diet (Purina Dog Checkers). The gross appearance of the liver of each animal was observed by laparotomy at the end of eight months, before the animals were placed on the stock diet. At autopsy, samples of all lobes of the liver and portions of other organs were fixed in Bouin's solution or neutral formalin. Paraffin sections were stained routinely with hematoxylin and eosin, and a few were also prepared with Masson's trichrome stain, Mallory's aniline blue, iron, acid-fast stain, mucicarmine stain, periodic acid-Schiff method, and Sudan IV.

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Scholar in Cancer Research of the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council (1951-1955).

This study was supported in part by research grants from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service (Grant No. A-610) and from The Life Insurance Medical Research Fund. A preliminary report of some of this work was presented at the annual meeting of the American Association for Cancer Research in San Francisco, April 17, 1955.

Results

Growth Curves.—All animals on ethionine showed an initial loss of weight for about two weeks. This was followed, in the 0.25% ethionine groups, by a period of weight gain at a rate comparable to that of the controls. The weights of these animals then leveled off at about five to six months and subsequently declined again until the time of killing or death.

Gross Appearance.—A summary of the gross findings is presented in the Table. In the first experiment, the livers of the control rats on the basal diet without ethionine were essentially normal. Of the four rats maintained on the 0.25% ethionine diet, three had multiple neoplasms in the liver, measuring from 1 to 6 cm. in diameter. The tumors were grayish-white and soft and contained many yellow, opaque areas of necrosis. In two of the animals,

Neoplasms in Livers of Rats Fed Ethionine

Experiment No.	Animal	Sex	Duration, Mo.	Gross Changes		
				Carcinoma in Liver		Metastases
				Occurrence	Diameter of Largest Nodule, Cm.	
I	1	F	8	Multiple	6	Omentum, pancreas
	2	M	8	Multiple	1	None
	3	M	9	Multiple	5	Omentum, pancreas, esophagus, stomach, intestine, diaphragm
	4	F	9	None	--	None
II	1	M	8	Multiple	0.5	None
	2	M	8	Multiple	3	None
	3	M	8* 10	None Multiple	-- 1	None
	4	M	8* 10	Multiple Multiple	3 4.5	None Omentum, lungs, mediastinum, pleura
	5	M	8* 10	Multiple Multiple	3 4	None Omentum, lungs, mediastinum
	6	F	8* 10	None None	-- --	-- --
	7	F	8* 10	Multiple Multiple	3 4	None Omentum, pancreas, lymph nodes
	8	F	8* 10	Multiple Multiple	0.5 1.5	None Abdominal carcinomatosis, lungs, mediastinum
	9	F	8	Multiple	4	None
	10	F	8* 10	Multiple Multiple	2.5 3	None Lungs
Total Incidence†				12/14		7/14

* Examined by laparotomy.
† Number of animals showing change over total number of animals.

multiple metastatic nodules, up to 1 cm. in diameter, were present in the omentum, pancreas (Fig. 1), serosa of the stomach, lower end of the eosphagus, and small and large intestines. In the animals without distant metastases, the liver tumors were similar but considerably smaller, measuring from a few millimeters to 1 cm. in diameter.

In the second experiment, in 9 of the 10 rats, some of each sex, grossly visible liver neoplasms were found at autopsy. The tumors were multiple, measured from 1 to 5 cm. in diameter, and involved one or more of all lobes of the liver. All tumors were grayish-white and soft, with yellowish-white areas of necrosis. Five of the nine rats with neoplasms showed metastases. These involved most frequently the lungs and omentum. In one animal, the abdomen showed generalized carcinomatosis, as well as extensive metastases to both lungs, pleura, and mediastinum (Fig. 2). In another animal, death occurred at 10 months from massive abdominal hemorrhage, originating from a tumor nodule in the liver. Serous or serosanguineous fluid was found in the peritoneal cavity of several of the animals with tumors.

In both experiments, the non-neoplastic portions of the liver presented a coarsely or finely granular surface. On section, multiple nodules, up to 0.5 cm. in diameter, replaced much of the liver. These nodules were of two types. The majority were discrete and reddish-brown and had a color and

Fig. 1.—Carcinoma in the liver of a rat on ethionine for nine months. Multiple secondary nodules are present at the hilus of the spleen and in the pancreatic bed (below). The pancreas is atrophic and replaced by fat.

Fig. 2.—Generalized abdominal carcinomatosis and metastases to the lungs and mediastinal lymph nodes in a rat on ethionine for eight months, followed by ethionine-free diet for two months. Microscopically, the liver tumors were both of the liver-cell type and anaplastic. The metastases were predominantly anaplastic with islands of mucous glands, but some distant nodules were liver-cell carcinoma.

consistency closely approaching that of normal liver. A smaller number, of yellowish-brown, softer nodules, were also seen in most livers. These had a uniform appearance on section and were sharply circumscribed. No gross evidence of necrosis was seen in either type of nodule. Microscopically, both types were found to be non-neoplastic. Diffuse fibrosis was not observed in any of the livers. However, many had localized, sometimes elevated, white regions, which were firm and somewhat rubbery. These areas were not sharply circumscribed and frequently appeared to spread diffusely into the surrounding tissue. On microscopic examination, they were found to be non-neoplastic regions of cholangiofibrosis.

Other organs also showed gross changes. In the first experiment, the pancreas of all animals on ethionine was atrophic and replaced in large part by adipose tissue. In the second experiment, in which many of the animals were on a stock ethionine-free diet for the final two months before being killed, the pancreas was larger than in the first experiment, and even looked normal in a few instances. In both experimental groups, the testes were very small and soft. The kidneys in all the males, but not in the females, showed opaque yellow linear streaks, confined to the inner, aglomerular zone of the cortex.[4] The spleen was generally enlarged. The remaining organs showed no consistent pathologic changes.

Microscopic Appearance.—Tumors: The neoplasms were invariably multiple, with many large and small nodules scattered throughout the liver. Although most of the nodules were discrete, they were not encapsulated, and many showed invasion of the surrounding liver tissue. Blood-vessel invasion was frequently seen. In a few instances, direct extension of a liver tumor into the muscle of the diaphragm was observed. Central areas of necrosis were common in tumor nodules of all sizes.

In some regions the tumor cells were

aligned in orderly cords or plates, but elsewhere there was little or no obvious anatomic orientation. At the one extreme, the liver cells were arranged in columns separated by either collapsed or compressed thin, endothelial-lined channels (Fig. 3). Most of the cords were two or more cells thick, in contrast to the normal rat liver, with its predominantly one-cell thick plates.[5] Some tumors showed the same basic arrangement, but with the columns separated by dilated vascular spaces, which were sometimes remarkably wide. Areas of gland-like formations, resembling adenocarcinoma, were prominent in some tumors (Fig. 4), but obvious transitions from solid hepatoma to glandular hepatoma were frequently seen. Many of the tumors lacked any organizational pattern. Large and small single cells and syncytial masses of neoplastic cells were jumbled together, without apparent architectural arrangement (Fig. 5). One tumor was composed of an unorganized mass of individually separate, but fairly uniform, tumor cells (Fig. 6). Here and there within this tumor occasional well-formed gland-like or duct-like structures were present (Fig. 7). These structures were invariably lined by a single layer of flattened epithelium, and their lumens were filled with amorphous material, which stained positively with the mucicarmine and periodic acid-Schiff stains. In many instances the lumens also contained poly-

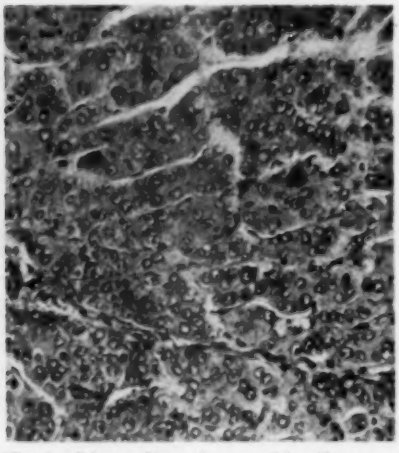


Fig. 3.—Liver-cell carcinoma with cells arranged in columns two or more cells thick, separated by endothelial-lined channels. The cells are fairly uniform in appearance. Hematoxylin and eosin; × 250.

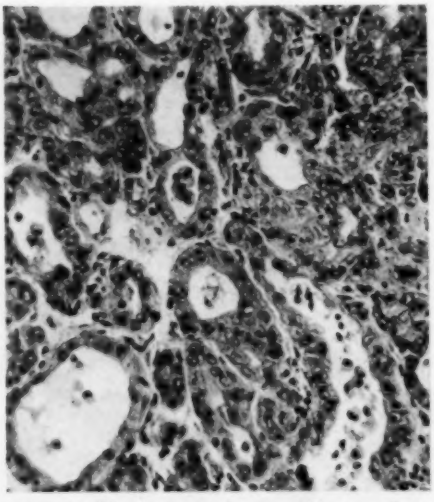


Fig. 4.—Liver-cell carcinoma with an adenomatous pattern. Desquamated tumor cells are present in many of the spaces. Areas such as this were negative for mucus with the mucicarmine and periodic acid-Schiff stains. Hematoxylin and eosin; reduced to 94% of mag. × 250.

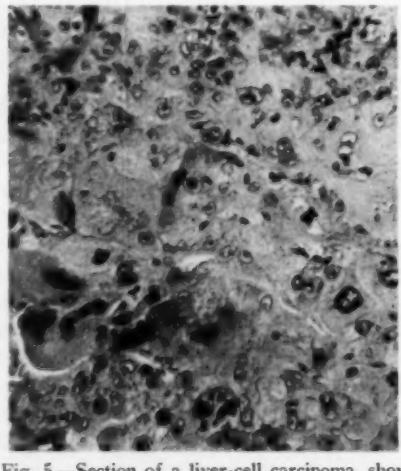


Fig. 5.—Section of a liver-cell carcinoma, showing many large irregular cells and some syncytial masses (giant cells). Hematoxylin and eosin; × 250.

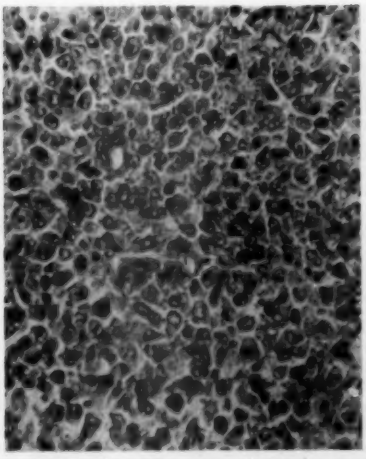


Fig. 6.—Section from an abdominal metastatic nodule in the rat shown in Figure 2. The cells are individually separated and are not arranged in any pattern. Many mitotic figures are present in the field. Hematoxylin and eosin; × 300.

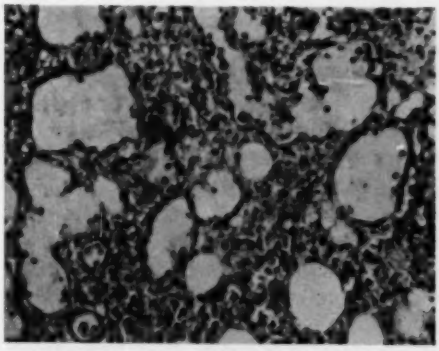
morphonuclear leukocytes. The positive mucous reaction in these glands was in sharp contrast to the negative reaction in the lumens of gland-like structures in all regions of the better-differentiated tumors.

A striking microscopic feature in all the tumors was the variation in cellular appearance and arrangement in different parts of single tumor nodules and in different nodules in the same liver. With the exception of the anaplastic tumor containing "mucous glands," transitions between the regions of different microscopic appearances were regularly observed. No bile canaliculi could be identified in any of the many sections examined.

In general, no correlation was found between the histologic appearance of the liver tumors and the presence or absence of metastases. The secondary neoplasms showed the same range of growth patterns observed in the primary tumors. However, the animal with the most highly anaplastic tumor in the liver had the most widespread metastases (Fig. 2).

Cytologically, the cells varied considerably. In some tumors most of the cells were quite uniform and resembled normal liver cells except for a greater nuclear-cytoplasmic ratio and a more basophilic cytoplasm (Fig. 3). Other tumors were composed predominantly of bizarre cells, with considerable variation in nuclear size, shape, and staining properties (Fig. 8). Striking cytologic variations were often present in

Fig. 7.—Section from same metastatic tumor nodule as that shown in Figure 6. Many gland-like spaces, lined by flattened epithelium, can be seen separated by anaplastic cells. These glands stained positively with mucicarmine and periodic acid-Schiff stains. Three fat cells are present in the lower right. Hematoxylin and eosin; reduced to 94% of mag. × 60.



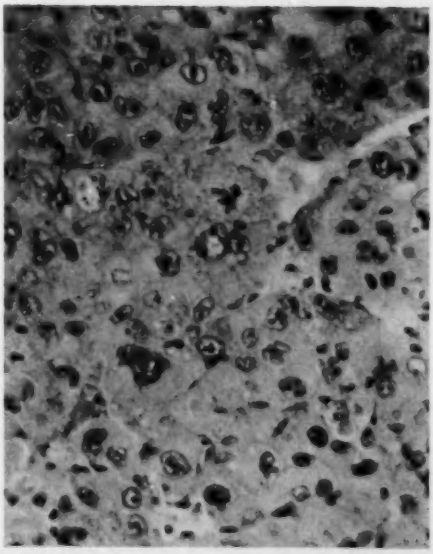


Fig. 8.—Section from a liver-cell carcinoma showing many large cells with irregular, dark-staining nuclei. Many mitotic figures, some abnormal, are present. Hematoxylin and eosin; reduced to 84% of mag. × 300.

different parts of the same tumor nodule. The nucleoli were prominent in most cells and were often multiple, even in otherwise well-differentiated cells. In hematoxylin-eosin preparations most of the tumor cells showed greater cytoplasmic basophilia than the non-neoplastic liver cells. Mitotic figures were frequent in all tumors. No cytologic differences were noted between metastatic nodules and the primary neoplasms.

Non-Neoplastic Portions of the Liver: In the liver surrounding the tumor nodules there was extensive proliferation of oval cells, believed to represent bile-duct epithelium. These cells had invaded or surrounded many of the preexisting liver lobules. Numerous regenerative nodules of hyperplastic liver cells were present, together with localized areas of cholangiofibrosis [6] and collections of bile ducts without accompanying fibrosis. Such changes are characteristic of chronic ethionine administration.[3]

The non-neoplastic liver nodules noted at the time of autopsy were basically of two kinds. The brown nodules, which resembled liver, were composed of islands of the original liver cells distorted by the progressive proliferation and differentiation of oval, duct-epithelial cells. Many isolated liver cells showed hypertrophy with increased nuclear and cytoplasmic basophilia. The paler nodules were islands of hyperplastic liver cells which probably had arisen from the preexisting parenchymal cells. Generally, they were easily distinguished from the "original" liver cells by the increased size of their cell cytoplasm, their compression of surrounding liver, and the general absence of oval cells along their sinusoids. They did not reproduce the lobular architecture of the liver. Some of these nodules were composed of very regular cells with uniform staining properties. Others, however, showed varying degrees of nuclear irregularity, with occasional large hyperchromatic nuclei (Fig. 9). In addition, there were nodules, with very basophilic cytoplasm, which resembled many of the frank carcinomas but were small and sharply circumscribed. Mitoses were not infrequently seen in these nodules. Whether these nodules are early carcinomas or immediate precursors of carcinoma could not be determined.

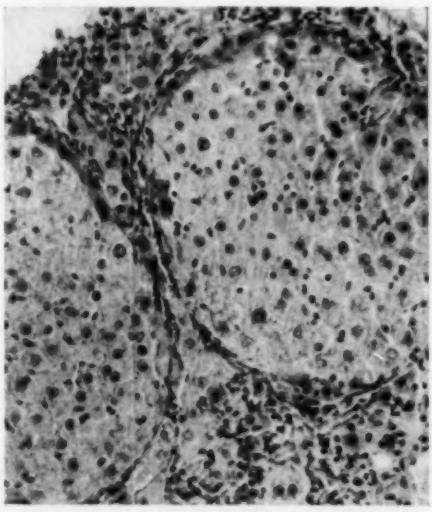


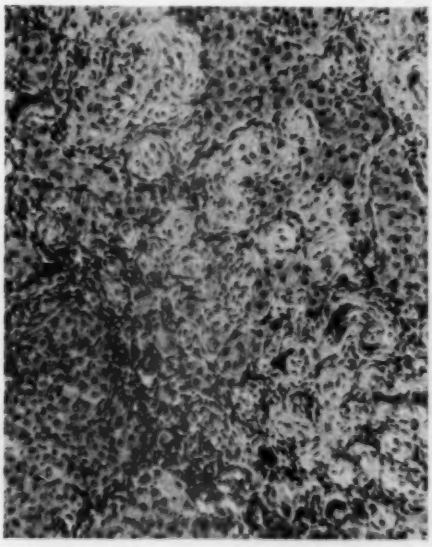
Fig. 9.—Two regenerative nodules showing some nuclear irregularity with hyperchromatism. Note the surrounding liver, with its proliferating oval cells and dissociated liver cells. There is some compression of this liver by the nodules. Hematoxylin and eosin; reduced to 84% of mag. × 200.

Scattered throughout the areas of greatest oval-cell proliferation were macrophages containing one of two kinds of pigment. One pigment was brownish yellow in color and stained positively for iron. The other pigment was a pale canary-yellow color and was negative for iron but positive with Sudan IV and acid-fast stains, suggesting its possible identity with ceroid pigment.[7] There was very little fibrosis outside areas of cholangiofibrosis.

These changes in the non-neoplastic liver are an exaggeration of the patterns seen in earlier stages of the reaction to ethionine and are difficult to understand unless the sequence of changes is followed. About one week following the beginning of ethionine feeding there occurs a proliferation of oval liver lobules.[3] These oval cells are probably

cells in or about the portal areas of the bile-duct epithelial cells. As ethionine administration is continued, these cells fan out radially from the portal triads between the liver-cell plates and the sinusoidal wall and between liver cells in the same plate to produce a progressive dissociation of the liver-cell lobule. This first stage is accompanied by atrophic and hypertrophic changes in the liver cells. The next phase is characterized by the appearance of increasing numbers of hyperplastic liver-cell nodules, which appear to arise from the original liver cells and produce progressive distortion of the liver architecture. While the nodular hyperplasia is proceeding, many of the oval cells begin to differentiate to produce bile-duct-like structures. These may take the form of collections of well-formed ducts, lined by a regular cuboidal epithelium, or, more commonly, of a localized area of cholangiofibrosis in which the ducts contain mucus and inflammatory cells and are surrounded by dense collagenous connective tissue. Owing to irregular coalescence of neighboring areas of differentiated oval cells, one may find islands of liver cells trapped in zones of cholangiofibrosis (Fig. 10), a finding sometimes misinterpreted as neoplastic invasion. Many of the ducts that develop in such lesions are lined by regular cuboidal epithelium, while others have deeply basophilic lining cells, many in mitosis. Although these changes superficially may resemble adenocarcinoma, we have never seen any real evidence of invasion or any metastases.

Fig. 10.—Islands of liver cells trapped in an area of cholangiofibrosis. Note the numerous ducts surrounded by fibrous tissue. Although most of the ductal lining cells are regular, some are intensely basophilic and have irregular nuclei. Transitions from undifferentiated oval cells into cholangiofibrosis are frequently present in such areas. Hematoxylin and eosin; reduced to 94% of mag. × 150.



Comparison of Findings at 8 and 10 Months.—In comparing the findings at 8 months, by laparotomy, and at 10 months, by autopsy, in Experiment II (Table 1), it is apparent that growth of the tumors in the liver and spread to other abdominal organs had occurred in the two-month period. Of the seven animals so examined, none had obvious abdominal metastases at the time of laparotomy, and four of these subsequently developed secondary tumors. This experiment also showed that the tumors were not dependent upon a continuous supply of ethionine, since they grew and spread after the animals were placed on a stock diet.

Comment

The most interesting conclusion from this study is that an analogue of methionine, which is capable of interfering, in acute experiments, with some of the intermediary metabolic roles of methionine,[3] is also able to induce malignant neoplasms in the liver. Whether cancer induction by ethionine is related to one or more of these disturbances in methionine metabolism is at present unknown. However, unpublished observations that adequate levels of methionine in the diet can prevent the pathological changes induced by the chronic administration of ethionine are compatible with this hypothesis.

The results of this study also show that the tumors induced by ethionine are autonomous, as is demonstrated by the fact that

growth of the tumors continues and metastases may develop after ethionine has been withdrawn from the diet. Subsequent unpublished experiments have confirmed this observation and have shown that feeding ethionine for only four months, at the end of which time no tumors are apparent, will ultimately give rise to liver cancer, six to seven months after the beginning of the experiment.

The hepatic tumors so far observed with ethionine have all been of the liver-cell type, with the exception of one anaplastic neoplasm, which contained islands of well-differentiated duct-like spaces containing mucus. The histologic characteristics of the liver-cell carcinomas were the same whether they were induced by ethionine, by 4-dimethylaminoazobenzene (butter yellow), or by one of the butter-yellow derivatives * or by 2-acetylaminofluorene.† This is not surprising in view of the fact that ethionine and all these other hepatic carcinogens produce histologically identical early hepatic lesions.[3]

Several classifications of experimental liver neoplasms have been proposed.‡ The liver-cell carcinomas in the rats fed ethionine reproduced the various morphologic types recognized in these classifications. However, in our studies, with the exception of the one anaplastic tumor, the tumors frequently showed transitions from one histologic type to another. Also, no correlation was observed between the histologic appearance and the presence or number of metastases. One must therefore conclude that the histologic or cytologic characteristics of these neoplasms did not reflect important differences in biological behavior and that within this group any classification based upon morphology alone would not have great practical value. Price and associates [14] have expressed a similar opinion.

Addition of ethionine to the growing list of hepatic carcinogens is of no great importance in itself. However, a good deal of information is available on the early biochemical effects of ethionine on the liver. This makes ethionine a potentially useful tool in studying the metabolic derangements during liver carcinogenesis. The known interference with methionine metabolism by ethionine,§ the suggested disturbance in nucleotide balance,[24] and the incorporation of ethione into liver protein [25] offer interesting points of attack for such studies. As indicated by Miller and Miller,[26] the possible analogy between ethionine incorporation into protein, presumably with the formation of "abnormal" proteins and the protein binding of butter yellow and its derivatives [27] and of 2-acetylaminofluorene,‖ is worthy of further consideration and study.

Summary

Carcinoma of the liver with invasion and metastasis has been observed in rats fed semisynthetic diets containing 0.25% DL-ethionine for periods of eight to nine months. Among 14 animals, 12 developed carcinoma of the liver and, of these, 7 had metastases. The tumors increased in size and metastasized after the ethionine was removed from the diet. Eleven of the tumors were of liver-cell type and resembled those reported following the administration of other hepatic carcinogens, such as butter yellow and its derivatives and 2-acetylaminofluorene. Transitions between solid and adenomatous patterns were frequently observed. In addition to the liver-cell tumors, one rat developed an anaplastic, metastasizing neoplasm which contained islands of gland-like structures filled with mucus. Since considerable information is already available about the metabolic effects of ethionine, it is suggested that this antagonist of methionine may be a useful tool in studies on the pathogenesis of carcinoma of the liver.

Mr. Tom Breaux and Mr. Jim Rush gave technical assistance during this study.

* References 6, 8-15.

† References 16, 17.

‡ References 6, 8, 9, 11, 12.

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Functional Disturbances of Respiration in Disorders of the Lung

Pathologic Considerations

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Numerous reports have appeared regarding the relationship of physiologic and pathologic changes in pulmonary diseases. However, many of these reports are aimed dominantly at either the pathologic or the physiologic aspect. This probably results from the fact that few pulmonary specialists are familiar with both pathologic and physiologic aspects of such diseases. Accurate correlation of these features of pulmonary disease has been difficult for a number of other reasons. In many instances it is difficult to interpret histologic changes in the diseased lung with regard to etiology and pathogenesis.

Tests of pulmonary function likewise are subject to limitations. They do not indicate the function of a small part of the lung but, rather, concern the pulmonary tree as a unit. Furthermore, these tests do not reveal the etiologic features of a disease but demonstrate how that disease affects function in a given patient. Because of the wide normal range of values for various tests of pulmonary function, a relatively large portion of the bronchopulmonary system must be involved before impaired function is evident. A small tumor obstructing a major bronchus is of much greater functional significance than is a peripheral tumor of similar size. Serial tests of pulmonary function in a patient may be of assistance because relatively small departures from the results of previous tests are of more significance than are slight deviations from the predicted normal values in a single test. The recent wider application of therapeutic surgical procedures in diseases of the lung has stimulated the investigation of pulmonary function.

Disorders of pulmonary function generally have been divided into three categories, namely, ventilation, diffusion, and perfusion (circulation). This division is understandably arbitrary, because each of these subdivisions is related to and dependent on the others. In any consideration of the pathologic entities that alter the various aspects of pulmonary function, it is likewise difficult to classify the disease arbitrarily as to the type of altered function. For instance, chronic fibrocaseous tuberculosis may alter pulmonary function by all three mechanisms. Tuberculosis of the lungs may alter pulmonary ventilation through involvement of the major or minor airways by producing a stricture of the bronchus. Changes in diffusion of gases may occur in chronic pulmonary tuberculosis as the result of parenchymal involvement. Vascular changes in the involved portion of the lung are also well recognized; thus, pulmonary perfusion may be altered.

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From the Section of Surgical Pathology (Dr. McDonald) and the Section of Medicine (Dr. Miller), Mayo Clinic and Mayo Foundation. The Mayo Foundation, Rochester, Minn., is a part of the Graduate School of the University of Minnesota.

Disorders of Ventilation

Disorders of ventilation are classified in Table 1.

TABLE 1.—*Disorders of Pulmonary Ventilation*

Obstruction to major airway
Tracheobronchial tumors
Inflammatory strictures
Broncholithiasis
Mucous plugs
Bronchiectasis
Obstruction to minor airway
Asthma
Bronchitis and mucous plugs
Emphysema
Some pneumoconioses
Compression of lung
Deformity of thoracic wall
Space-occupying lesion within the thorax
Miscellaneous (fetal atelectasis; congenital abnormalities of bronchopulmonary tree)

Obstruction to Major Airways.—Movement of gases into and out of the various portions of the pulmonary system may be impaired by a variety of lesions. Narrowing of a major airway increases resistance to the flow of gases and may lead clinically to stridor or a coarse wheeze. This turbulence is likely to be more pronounced with exercise, when the minute ventilation is greater, than it is at rest. Such patients occasionally are treated for bronchial asthma, only to be found later to have a localized benign or malignant tumor encroaching on the trachea or major bronchi.

No particular type of endobronchial lesion is regularly associated with this asthmatoid picture. While one patient with a given endobronchial lesion may have bouts of wheezing, another patient with a similar lesion may not have episodic wheezing. The reasons for this individual variation are not well understood. Accumulation of mucus at or distal to the site of stenosis may be a factor. Secondary spasm of the smaller airways may explain this phenomenon in some instances. Such a lesion was a fibrolipoma of the trachea in a 37-year-old man (Fig. 1). This tumor was removed locally, and the patient was rid of his asthmatic symptoms. If the localized lesion reaches such a proportion that only inspired air passes the obstruction, the mass acts as a ball valve. Obstructive emphysema then may develop in the pulmonary parenchyma distal to the lesion. This portion of the lung shows increased radiolucency on roentgenograms and fails to become radiopaque during expiration. Hence, roentgenograms taken at both inspiration and expiration are of diagnostic importance.

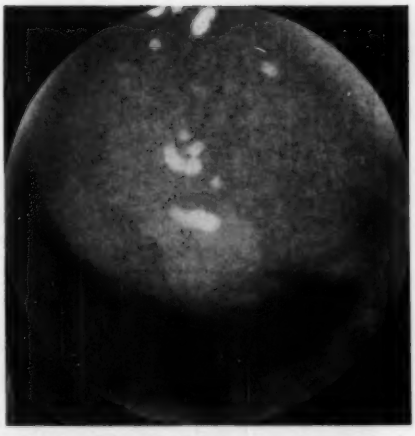

Fig. 1.—Polypoid fibrolipoma of the bronchus as seen bronchoscopically.

Chronic obstruction of the bronchi commonly produces a lesion that is best referred to as obstructive pneumonitis. Many physicians term this lesion "atelectasis," but we believe this is incorrect. The first phase of obstructive pneumonitis [17] consists of an outpouring of edema fluid into the pulmonary alveoli. Loss of volume of the involved parenchyma may not occur in this stage (Fig. 2). The affected alveoli become infected, with subsequent infiltration by polymorphonuclear leucocytes and other cells characteristic of inflammation. As organization progresses, the involved parenchyma undergoes fibrosis and contracts in volume (Fig. 3). In this later stage endarteritis is present in the pulmonary arteries and arterioles, thus reducing the size of their lumina (Fig. 4). Such changes also are seen in other pulmonary conditions associated with extensive destruction of pulmonary tissue of a chronic nature; they

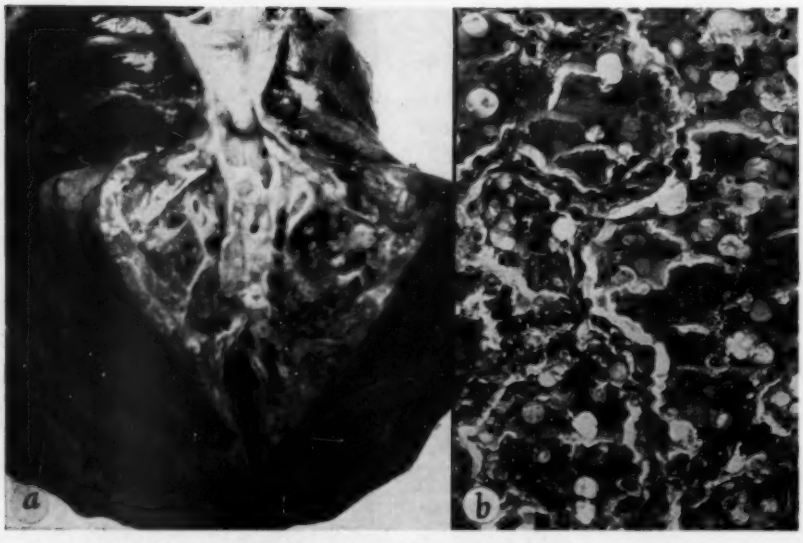

Fig. 2.—Early phase of obstructive pneumonitis caused by bronchogenic carcinoma. (*a*) The lung is voluminous and appears waterlogged. Reproduced with the permission of C. V. Mosby Company, St. Louis, from an article by J. R. McDonald and associates (*J. Thoracic Surg.* 18:97-112 [Feb.] 1949). (*b*) Edema fluid in the pulmonary alveoli. Hematoxylin and eosin; × 250.

probably constitute a type of physiologic endarteritis.*

The thoracic roentgenogram of a patient with carcinoma of the bronchus is shown in Figure 5. Table 2 summarizes some of the preoperative findings in tests of pulmonary function on this patient. The vital capacity was 54% of the predicted normal, as was anticipated with one lung almost completely excluded from the pulmonary ventilation. The alveolar content of nitrogen after the patient breathed "100%" oxygen for seven minutes was 0.7% (normal is less than 2.5%), indicating normal distribution of gases in the pulmonary parenchyma that was still ventilated. The arterial oxygen saturation was 88% at rest; it increased to 100% when the patient breathed "100%" oxygen and did not decrease significantly after step-test exercise. This suggested that some pulmonary arterial blood was perfusing the nonventilated, right lung. The maximal breathing capacity was not determined because of grossly bloody sputum. The patient had no increase in dyspnea after operation.

Complete obliteration of the bronchial lumen is not a prerequisite for obstructive pneumonitis. At times the bronchial lumen may be widely patent, but if a segment of bronchial wall is infiltrated, the elimination of secretions is interfered with and obstructive pneumonitis ensues (Fig. 6). The infiltration is usually carcinomatous. This so-called physiologic obstruction is seen elsewhere in the body,[8] as in the bile duct and ureter.

As already indicated, "atelectasis" is a commonly misused designation. The term is applied to a number of conditions that have widely varying histologic characteristics and genesis. It is most accurately applied to that condition in the fetus in which the lung fails to expand. It is also used for compression atelectasis, in which the pul-

* Samper, R.: Vascular Changes Secondary to Carcinoma of the Lung, Thesis, Graduate School, University of Minnesota, 1946.

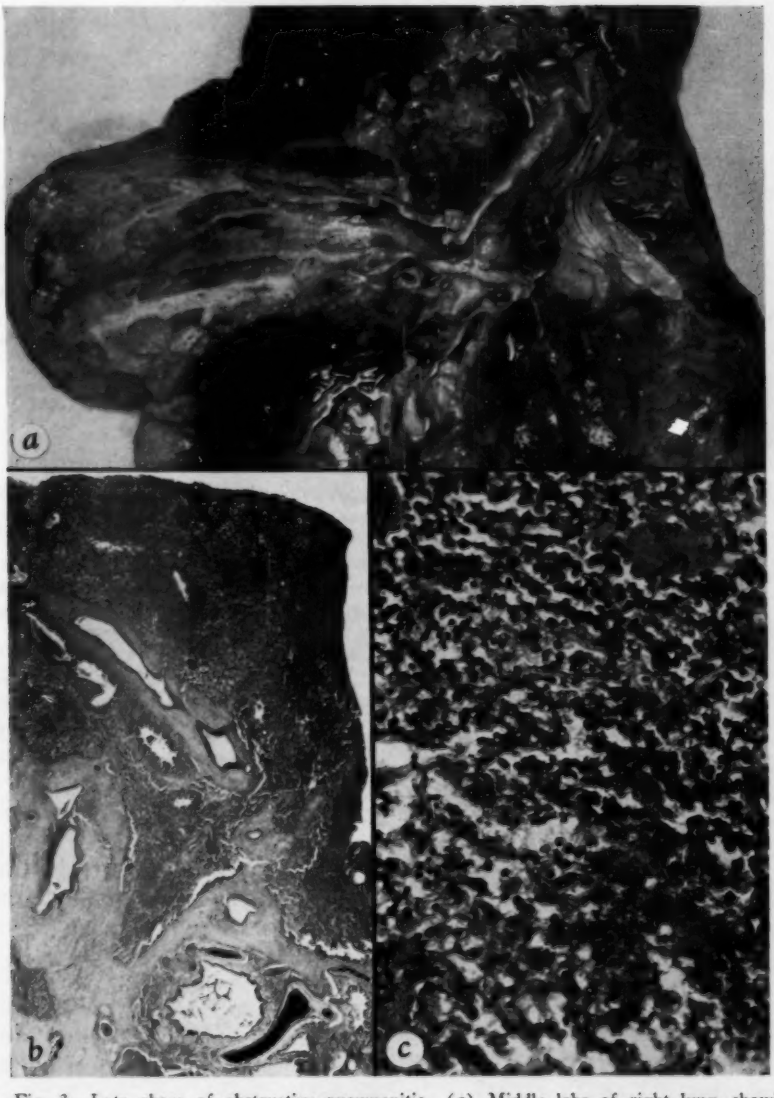

Fig. 3.—Late phase of obstructive pneumonitis. (*a*) Middle lobe of right lung, showing pronounced contracture. The cause of obstruction was carcinoma of the bronchus. (*b*) Section demonstrating lack of air in the parenchyma and the patent, partially dilated bronchi. Hematoxylin and eosin; × 2. (*c*) Contracture as the result of obstruction. The pulmonary alveoli are obliterated; fibrosis and inflammatory exudate are pronounced. Hematoxylin and eosin; × 250.

monary parenchyma is compressed by extrapulmonary mass. In addition, it is used in connection with varying forms of bronchial obstruction. Amberson † has stated:

† Amberson, J. B., quoted by Shields.[3]

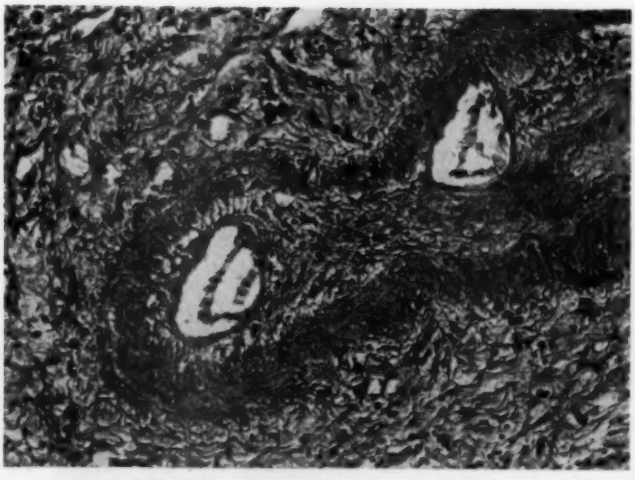

Fig. 4.—Obstructive pneumonitis in the contracted phase caused by carcinoma of the bronchus. Note physiologic endarteritis of a pulmonary artery, resulting in great reduction of size of lumen. Hematoxylin and eosin; × 125.

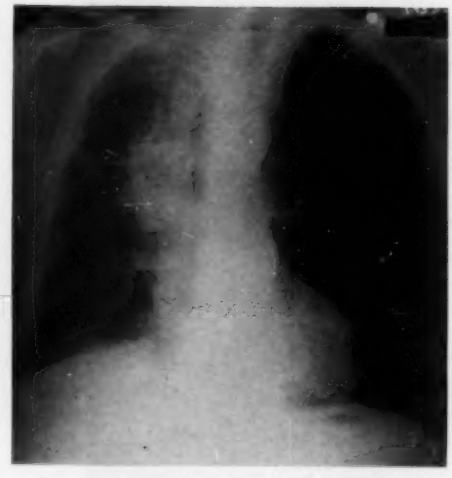

Fig. 5.—Preoperative roentgenogram of a 68-year-old man who had bronchogenic carcinoma obstructing the airways to the entire right lung.

TABLE 2.—*Tests of Pulmonary Function in a Patient with Bronchogenic Carcinoma*

Test	Estimated normal	Values in Patient
Vital capacity, liters	4.8	2.59
Residual volume, liters	1.6	1.67
Total lung capacity, liters	6.4	4.26
Airtrapping	None	Questionable
Arterial oxygen saturation, %	95-98	88

I think the diagnosis of atelectasis in the specific sense is usually erroneous, because as a rule there is little or no collapse of the alveoli, but, rather, an accumulation of edema fluid, which is often soon followed by pneumonia. . . . The important concept, which is fundamental, is that collapse of the alveoli in itself does not predispose to pneumonia, whereas edema always does.

Acute obstruction of the bronchus is commonly seen postoperatively. The offending agent is a plug of mucus. Evidence of such obstruction, as denoted by loss of volume, is seen roentgenographically. Histologic verification is seldom available in this stage because it is apparently transient. The collapsed alveoli soon are filled by edema fluid, polymorphonuclear leucocytes, and mononuclear cells. This stage is manifest clinically by a febrile course. According to Spain,[25] acute obstruction of the bronchus in dogs leads to varying degrees of atelectasis, which is not at all consistent. He explains this on the basis of collateral respiration, which was described by Van Allen and Lindskog.[30] They showed that parts of the lung communicate with other parts of the lung, so that air from one part of the bronchial tree can pass into another part of the pulmonary parenchyma. They were uncertain as to where in the lung this communication takes place.

Another form of bronchial obstruction is seen in essential bronchiectasis, in which

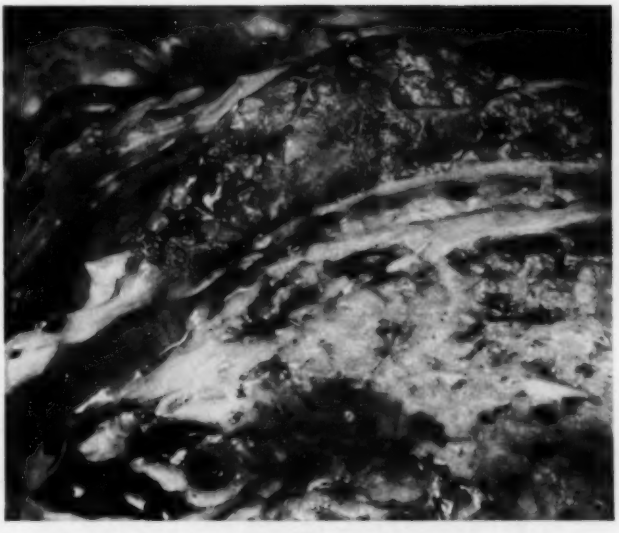

Fig. 6.—Squamous-cell carcinoma of the lingular division of the left upper lobe. The thickened bronchus indicates the site of the carcinoma. The lumen of the bronchus is widely patent, with obstructive pneumonitis in the distal part of the parenchyma.

destruction and fibrosis of the bronchial wall can be demonstrated histologically.‡ The amount of elastic tissue in the wall of the bronchus is decreased and splinting of the wall occurs. The secretions in the involved parenchyma are retained and the bronchi dilate. Repeated bouts of pneumonitis frequently lead to varying degrees of parenchymal organization and fibrosis. It is by this mechanism that chronic pulmonary insufficiency occurs. The amount of parenchymal destruction and fibrosis seen in surgically resected specimens has been greatly reduced in recent years, presumably because of the use of antibiotics. The exact genesis of this disease is not clear. Bronchial dilatation of various degrees is one of the consequences of many types of bronchial obstruction.

Obstruction to Minor Airways.—This type of obstruction occurs in a variety of conditions. A patient with an attack of bronchial asthma represents one of the most classic abnormalities of this type. Bronchospasm is associated anatomically with hypertrophy of the bronchiolar musculature and mucous glands and with plugging of the minor airways by inspissated mucus (Fig. 7*a*). Simple episodic asthma seldom leads to permanent pulmonary insufficiency. Prolonged intractable asthma with recurrent infection may be complicated by emphysema, fibrosis, hypoxia, and polycythemia. With these complications, pulmonary hypertension and cor pulmonale ensue. Chronic bronchitis often may be associated with clinical, physiologic, and pathologic features resembling those of bronchial asthma. However, evidence of chronic infection is prominent. Chronic bronchitis may be related to microbial or viral infection or exposure to various noxious physical agents, such as dusts or chemicals. Chronic bronchitis is considered to be "nonspecific."

Diffuse pulmonary emphysema may occur as a complication of one of these conditions associated with stenosis or obstruction of small airways. The "essential" lesion in primary diffuse obstructive emphysema has been considered to be chronic bronchiolitis and peribronchiolitis, resulting in luminal narrowing and distention of distal alveolar spaces.[27] There is little evidence of selective loss of elastic tissue in the alveolar walls. The alveolar spaces are greatly dilated, and the septa are attenuated. Large bullae may be formed as a result of rupture of many

‡ Ginsberg, R. L.: Personal communication to the authors.

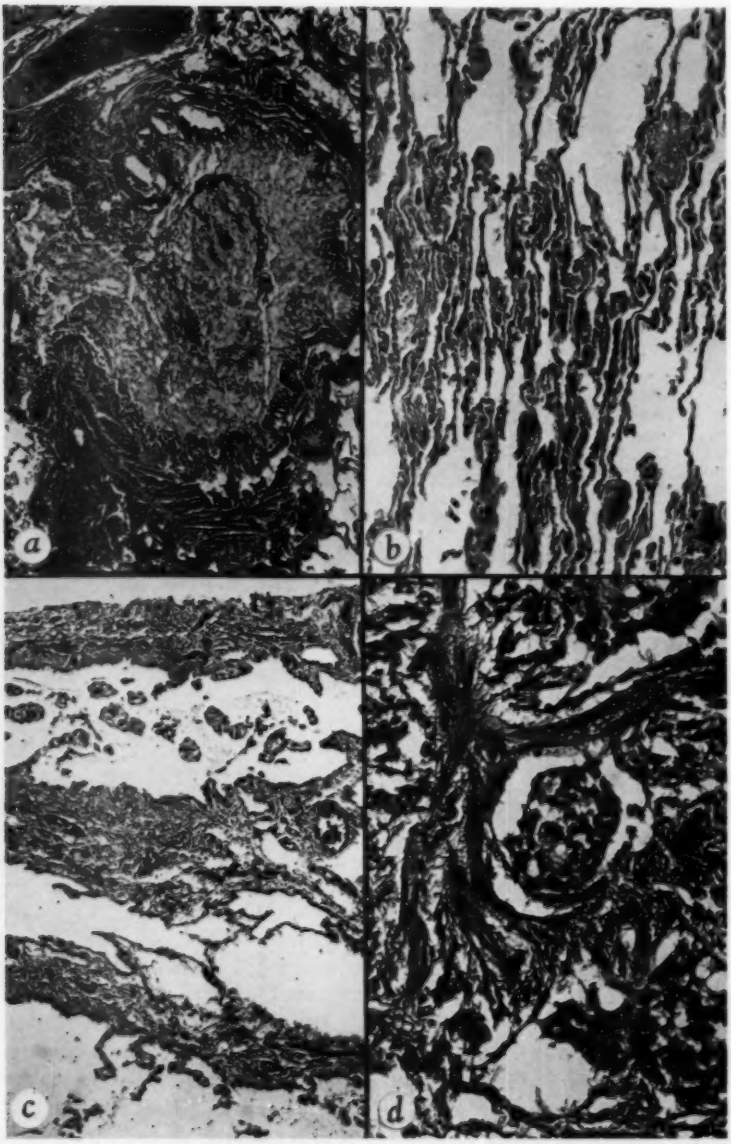

Fig. 7.—(*a*) Medium-sized bronchus containing inspissated mucus in bronchial asthma. Muscle in wall of bronchus is hypertrophied. Hematoxylin and eosin; × 40. (*b*) Compression atelectasis. Note collapsed alveoli and lack of inflammatory change. Hematoxylin and eosin; × 100. Reproduced with the permission of C. V. Mosby Company, St. Louis, from an article by J. R. McDonald and associates (*J. Thoracic Surg.* 18:97-112 [Feb.] 1949). (*c*) Emphysematous blebs beneath pleural surface. Hematoxylin and eosin; × 60. (*d*) Chronic interstitial fibrosis. Note pronounced fibrous thickening of pulmonary alveoli. Hematoxylin and eosin; × 175.

septa. Capillaries are widely spaced. Pulmonary hypertension follows usually the development of hypoxia. The late stages of emphysema are characterized by pulmonary hypertension, cor pulmonale, congestive cardiac failure, and death unless the course is interrupted by acute respiratory infection.

Compression of Lung.—Compression of normal pulmonary parenchyma may lead to pulmonary insufficiency. It is remarkable, however, how much parenchymal compression can occur with little or no respiratory symptoms as long as the tracheobronchial passages are not greatly compromised. This is particularly true if the change occurs over a long period. Pronounced kyphoscoliosis and large tumors of the pleura, thoracic wall, or mediastinum may be asymptomatic, depending on the factors just mentioned. Determinations of pulmonary volume may reveal abnormally low values if the compression is great enough, even though symptoms are absent. The pulmonary lesion may be termed "compression atelectasis"; it consists of apposition of the alveolar walls in the absence of inflammatory change (Fig. 7*b*).

Emphysematous bullae or blebs are seen occasionally unassociated with generalized emphysema (Fig. 7*c*). The genesis of these lesions is unknown, but they usually occur in younger persons than do those emphysematous bullae associated with generalized emphysema. Pulmonary insufficiency may result from compression of apparently normal adjacent parenchyma. The frequent complication of spontaneous pneumothorax may lead to sudden respiratory distress.

Miscellaneous Conditions.—A number of unusual lesions, such as fetal atelectasis or hypoplasia or agenesis of a lobe or a lung, obviously may reduce pulmonary volume and, to some extent, the ventilatory capacity.

Disorders of Diffusion

Disorders of diffusion are classified in Table 3. Diffusion of gases between the blood and gas phases in the lung is, of course, dependent on adequate ventilation of alveoli with inspired air and perfusion of these alveoli with mixed venous blood from the pulmonary artery. Diffusion of oxygen and carbon dioxide then depends on the thickness and the chemical and physical nature of the alveolar capillary membrane, as well as the total surface area of that membrane.

TABLE 3.—*Disorders of Pulmonary Diffusion*

Thickened alveolar membrane
Chronic interstitial fibrosis
Acute interstitial fibrosis (Hamman-Rich)
Scleroderma
Uremia and alveolar membrane of newborn
Loss of pulmonary parenchyma (loss of diffusing surface)
Excessive pulmonary resection
Pathologic loss of pulmonary parenchyma
Malignant tumors
Alveolar-cell tumor
Granulomatous diseases (such as Boeck's sarcoid, berylliosis, silicosis)
Bronchopneumonia
Lobar pneumonia
Lipoid pneumonia
Emphysema

Thickened Alveolar Membrane.—A thickened alveolar membrane has been described in a number of conditions, such as chronic interstitial fibrosis (Fig. 7*d*) and so-called progressive interstitial pneumonitis, or the Hamman-Rich syndrome.§ The last mentioned condition is characterized by a rather rapid and relentless course, limited to several months. The differentiation of chronic interstitial fibrosis and the Hamman-Rich syndrome is not clear-cut and appears to be based on the clinical course rather than any specific histologic features or etiologic factors. A hyaline membrane lining the alveolar walls has been described in the latter syndrome but is not always present.

Data in a case of progressive interstitial pneumonitis are summarized in Table 4. These findings indicate normal ventilation but severe reduction in diffusion of oxygen. The roentgenogram of the thorax in this case is shown in Figure 8*a*. Pulmonary tissue removed at biopsy is shown in Figure 8*b*.

When the cause in a given case is indeterminate, the case is usually placed in one of the two categories just mentioned.

§ References 11, 12, 21, 24.

TABLE 4.—*Tests of Pulmonary Function in a Patient with Progressive Interstitial Fibrosis*

Test		Estimated normal	Values in This Patient
Vital capacity, liters		4.3	4.38
Residual volume, liters		1.5	1.6
Maximal breathing capacity, liters/min.		112	117
7-minute alveolar nitrogen, %		Less than 2.5	0.7
Arterial oxygen saturation, %	Rest	96-98	85.5
	Breathing "100 per cent" oxygen	100	100
	Exercise (air)	95-98	69

Actually, chronic interstitial pulmonary fibrosis is a fairly rare pathologic entity.‖

‖ References 16, 20.

When the cause is known, the disease is then named etiologically. Irradiation pneumonitis is a common cause of interstitial fibrosis. Doniach [6] has described an alveolar membrane lining the lungs in a case of uremic pneumonitis and contributing greatly to pulmonary dysfunction. A hyaline membrane also has been described lining the alveolar spaces of the lungs of newborn infants (Fig. 9*a*). This is considered to differ from fetal atelectasis.[22]

Certain industrial diseases, such as acute berylliosis and bauxite poisoning,[26] have been reported to produce diffuse interstitial fibrosis. More frequently, the pneumoconioses appear to be characterized by diffuse nodular lesions in the smaller airways or alveoli. Certain antihypertensive drugs recently have been reported to cause a clinical syndrome resembling acute interstitial fibrosis [7] or lupus erythematosus.[9] Stopping administration of the drug in question was

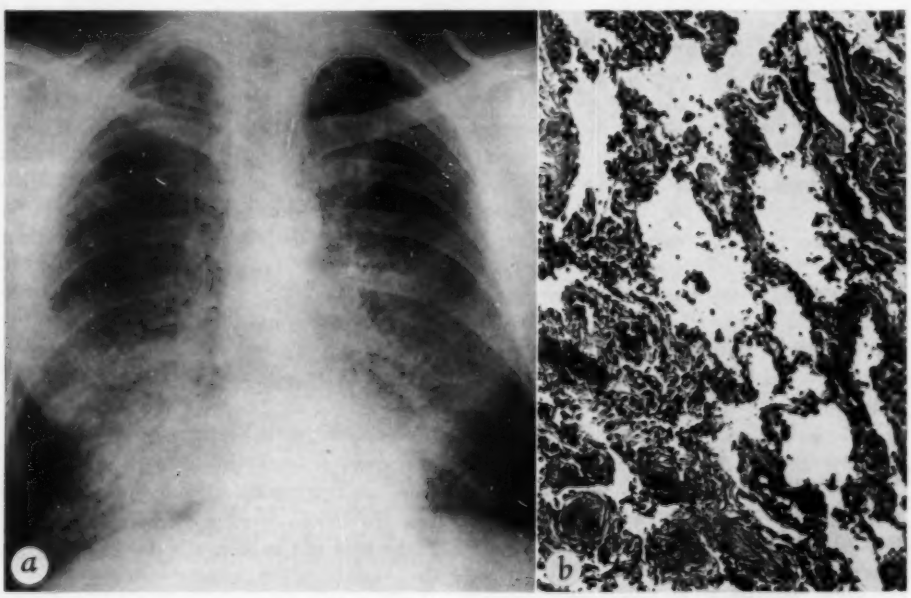

Fig. 8.—Progressive interstitial pneumonitis in a 47-year-old man with a three-month history of progressive dyspnea, cyanosis, and polycythemia. All bacteriologic studies were negative. (*a*) Posteroanterior roentgenogram. (*b*) Tissue removed from right upper lobe for diagnosis. Note pronounced thickening of interalveolar septa, with hyaline membrane lining alveolar spaces. Hematoxylin and eosin; × 120.

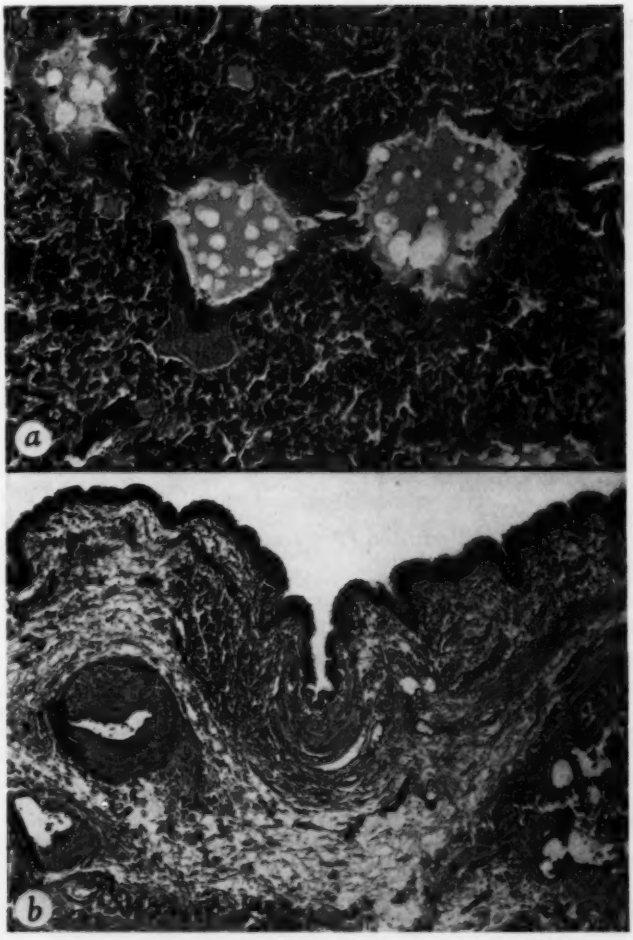

Fig. 9.—(*a*) Hyaline-membrane disease of the newborn. Note hyaline membrane lining pulmonary alveoli. Hematoxylin and eosin; × 250. (*b*) Bronchus in bronchiectasis. Note end-arteritis in large bronchial artery in wall of bronchus. Hematoxylin and eosin; × 55.

followed by reversal of the clinical syndrome in some instances. Thickening of alveolar septa was reported in some cases in which death occurred. Whether these changes represented a diffuse collagen reaction or a manifestation of congestive cardiac failure is not known. Mallory has expressed the opinion that long-standing congestive cardiac failure may result in histologic changes resembling those of interstitial pulmonary fibrosis.

Scleroderma has been reported to be associated with interstitial fibrosis [10] and the clinical syndrome of "alveolocapillary" block, or impaired diffusing capacity. Other members of the group of "collagen" diseases also may be associated with thickening of alveolar septa, but vascular lesions usually appear to be more prominent.

Loss of Pulmonary Parenchyma.—Reduction in pulmonary parenchyma leads to reduction in the area of the diffusing surface. Excessive pulmonary resection impairs diffusing capacity, even though the remaining alveolar-capillary membrane is normal. Replacement of the parenchyma by pathologic tissue in various types of diffuse nodular pulmonary disease also reduces the diffusing capacity and alters the "viscoelastic" properties of the lung. Diffuse

granulomatosis (tuberculosis, fungous disease, sarcoidosis), metastatic malignant lesions, alveolar-cell tumor, pneumoconiosis, bronchopneumonia, and lobar pneumonia might be placed in this category. Long-standing diffuse nodular disease of the lung also reduces the capacity of the pulmonary vascular bed, and the terminal phases of many of these non-neoplastic conditions may be cor pulmonale and congestive cardiac failure.

Emphysema.—Diffuse pulmonary emphysema, whether occurring secondary to bronchiolar involvement by one of the aforementioned processes or as a "primary" entity, results in loss of pulmonary parenchyma, with reduced diffusing capacity.

Disorders of Perfusion (Pulmonary Circulation)

A number of conditions cause disease of the pulmonary vasculature (Table 5). Pulmonary embolism, manifested by a massive

TABLE 5.—*Disorders of Pulmonary Perfusion*

Pulmonary embolism
Arteriosclerosis
Collagen diseases
Polyarteritis nodosa
Lupus erythematosus
Parasites (schistosomiasis)
Vascular anomalies

single embolus or by multiple small emboli, probably represents the commonest pure vascular disorder impairing pulmonary circulation. Many instances of so-called primary pulmonary hypertension have been found at necropsy to be secondary to multiple minute pulmonary emboli.[19] Emboli of amniotic fluid occurring during delivery can produce death[28]; if the patient survives, pulmonary arteriosclerosis or pulmonary hypertension may ensue.[15] Many instances of so-called lymphogenous spread of carcinoma to the lung probably represent, in reality, myriads of small pulmonary emboli, as suggested by Ballantyne ¶ in a study on pulmonary embolization by bronchogenic carcinoma.

The "collagen" diseases, particularly periarteritis nodosa and disseminated lupus erythematosus, frequently are associated with pulmonary vascular lesions. Pulmonary hypertension may result in some instances. Primary systemic amyloid disease and amyloid disease of the heart frequently are associated with amyloid deposits in the pulmonary vascular tree.[5] Primary pulmonary arteriosclerosis is extremely rare but probably does occur. This diagnosis is made by exclusion when thorough anatomic studies fail to reveal a causative factor.

Pulmonary arteriovenous communications present a different type of disorder of perfusion.[3] Small isolated fistulas usually are of interest chiefly because they present indeterminate roentgenologic shadows requiring exploratory thoracotomy; the resultant physiologic abnormalities are usually minor and produce little or no disability.[29] Larger or multiple pulmonary arteriovenous fistulas are associated with pronounced right-to-left shunts with hypoxemia but with no pulmonary hypertension or cardiac enlargement.[4]

Another type of vascular anomaly is seen in bronchopulmonary sequestration.[2] In this condition, the lung, usually a lower lobe, is supplied by an anomalous artery from the systemic circulation, frequently the aorta. In one such case,[13] evidence of communication between this anomalous artery and the pulmonary artery was absent. Such a condition seldom produces symptoms because there is no perfusion of the segment by pulmonary arterial blood.

Vascular changes occur as secondary manifestations in many diseases of the lungs. Numerous investigators have demonstrated these changes by various techniques. Wood and Miller[31] demonstrated communications between the bronchial and the pul-

¶ Ballantyne, A. J.: Incidence of Venous Involvement in Cancer of the Lung, Thesis, Graduate School, University of Minnesota, 1956.

monary arterioles by injection with radiopaque material. In numerous pulmonary diseases, these communications become prominent and the bronchial arteries are dilated and tortuous (Fig. 9*b*). Liebow and associates, by means of injection and corrosion techniques, confirmed these findings and extended the investigations into the fields of venous communications and bronchial musculature.[14] Roosenburg and Deenstra[23] have demonstrated the functional significance of these investigations.

Physiopathologic Correlation

It should be emphasized again that clear-cut separation of disorders of ventilation, diffusion, and perfusion is impossible. Although a particular pathologic entity initially may impair one of these aspects of function, one or both of the other categories often undergo subsequent change.

Ventilation of the lungs is dependent on adequate airways. The rate at which gases can be moved in and out of the lungs is measured by several means, including the maximal breathing capacity, the fast vital capacity (the percentage of the total vital capacity exhaled in one, two, or three seconds) and the maximal midexpiratory flow (the rate of flow or duration of time of the middle half of the fast vital capacity). If the values obtained for these tests are below the predicted normal levels and the patient has cooperated adequately, anatomic or functional obstruction of the airway is present. In patients who have bronchial asthma, these values may be significantly decreased only during attacks, while the subnormal values are more constant in patients who have emphysema or severe tracheal narrowing.

The standard vital capacity, although an important measurement, has long been recognized to have limited value because the factor of time is not present. Thus, the standard vital capacity could be normal in the presence of expiratory slowing, whereas one of the tests that involve a time factor may reveal an abnormality. If obstruction of the airway is so great as to cause persistent trapping of air or obstructive pneumonitis, the standard vital capacity also will be decreased. If air is trapped distal to the obstruction of a major or minor airway, the total lung volume remains essentially the same, the residual volume increasing at the expense of the vital capacity. Pulmonary emphysema presents a classic example of this type of defect. If obstructive pneumonitis occurs distal to the obstruction, the total lung capacity very likely becomes diminished.

Disorders of diffusion of gases may occur with little alteration in ventilation or perfusion. This occurs classically in diffuse interstitial fibrosis. The maximal breathing capacity may be entirely normal. The vital capacity may or may not be diminished, but the arterial oxygen tension is low. If patients with this condition breathe "100%" oxygen, the arterial oxygen saturation increases to normal. In disorders primarily affecting ventilation, the arterial blood will not be normally oxygenated if part or all of the alveolar spaces are underventilated so that the oxygen tension decreases significantly and the alveoli are perfused with mixed venous blood. This does not indicate, however, that diffusion is impaired. In this situation, the inhalation of "100%" oxygen corrects the arterial oxygen desaturation usually in a period of minutes, because the underventilated alveoli will attain a high oxygen tension.

Disorders of pulmonary circulation, with the exception of arteriovenous shunts, usually lead to pulmonary hypertension and cor pulmonale. If multiple small pulmonary emboli or primary pulmonary arteriosclerosis occurs, there is increased resistance to pulmonary blood flow. Ventilation to all alveoli may continue in a relatively normal fashion. Those alveoli with open pulmonary vessels and perfused with mixed venous blood will allow adequate oxygenation of the blood to occur. Alveoli to which pulmonary circulation is cut off are then hyperventilated, because less oxygen is ex-

tracted in relation to the ventilation than in the normally perfused alveoli.

Pulmonary arteriovenous fistula presents a different problem, which simulates right-to-left intracardiac shunts. In patients with this lesion, if the magnitude of the shunt is great enough, the arterial oxygen saturation fails to reach normal, even though the patient breathes "100%" oxygen. This condition is thus differentiated from that of diffusion barrier or hyperventilation with arterial hypoxemia.

Summary

Pulmonary dysfunction is classified as disorders of ventilation, diffusion, and perfusion. An attempt has been made to place certain pathologic entities into these categories. Strict delineation of a given disease under a single functional classification is impossible in that many diseases produce abnormalities in two or three of these categories.

Despite the numerous contributions in the field of anatomicophysiologic correlation of pulmonary diseases, much is not well understood. This field should form a fruitful source for future investigation.

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Histophysical Studies on Corpora Amylacea from the Human Spinal Cord

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Corpora amylacea are spherical, extracellular bodies measuring from several to 50μ in diameter, which are found in the brain, spinal cord, prostate, and lung. In the central nervous system these bodies are most commonly found in the subependymal, subpial, and perivascular regions. Their number increases in old age and in the presence of degenerative and encephalomalacic processes. Since Morgagni's first description, in 1723, and Virchow's publication,[7] in 1854, a number of morphological and histochemical studies have been done. Particular mention is made here only to the histochemical studies carried out by Steele, Kinley, Leuchtenberger, and Lieb,[6] published in this journal in 1952. One of the most recent contributions is the paper by Diezel,[2] in 1956. The histochemical investigations reported by this author show that corpora amylacea do not contain lipids, proteins, or nucleic acids. Therefore, they should not be considered to be degeneration products of myelin sheaths or cells. The histochemical studies, however, demonstrated that corpora amylacea are mainly composed of acid mucopolysaccharides of a relatively high degree of polymerization.

We here carried out histophysical measurements on corpora amylacea, obtained from a human spinal cord. The material was taken from the cord of a 53-year-old white woman with diabetes mellitus, fibrosis of the pancreas, benign nephrosclerosis, and acute pyelonephritis, who finally died of uremia. In the spinal cord a large number of corpora amylacea was present (Fig. 1).

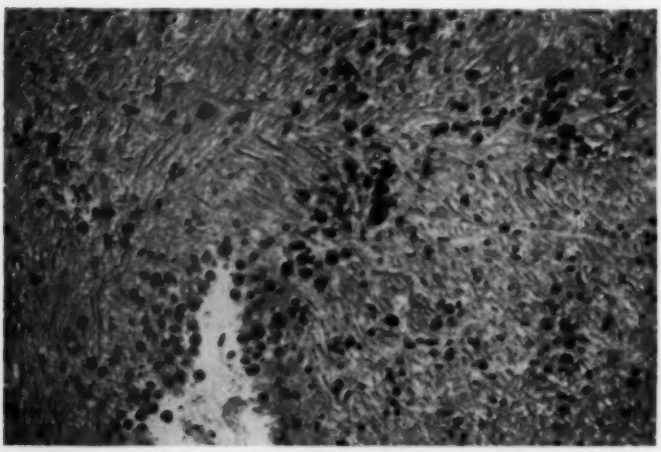

Fig. 1.—Corpora amylacea near the sulcus centralis of a human spinal cord. Hematoxylin-eosin stain; reduced to 58% of mag. × 90.

The refractive index, the mass concentration, and the mass itself were determined on about 60 cor-

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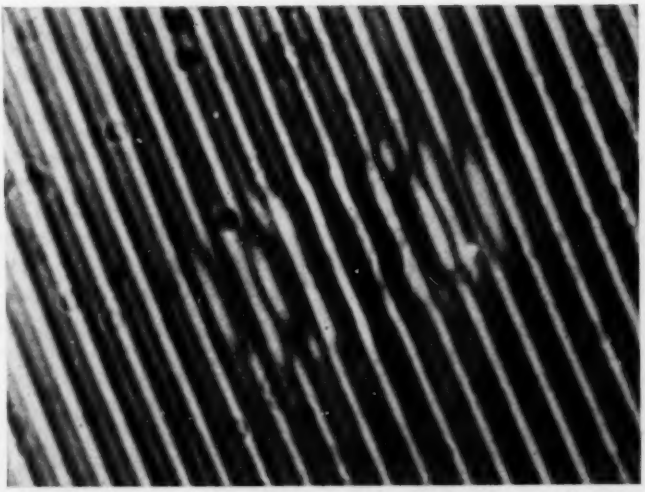

Fig. 2.—Refractometric pattern of a single corpus amylaceum, photographed with the schlieren grating microscope. Mag. × 800.

pora amylacea. This was done with the help of a microscopic schlieren arrangement which gives results similar to those obtained by an interference microscope, although its basic principle is quite different. In the final image, obtained by the schlieren microscope, a system of straight dark lines, called the background pattern, becomes visible. Optical inhomogenities within the specimen to be examined lead to characteristic deformities and disfigurations of this background pattern which are directly proportional to the refractive index, concentration, and anhydrous mass of the object. A theoretical analysis of the optical principles involved in this method and the details of the technique have been published previously.*

Figure 1 shows a number of corpora amylacea in an area of encephalomalacia near the sulcus centralis of the spinal cord of this patient. The section is stained with hematoxylin-eosin to show the histological appearance of these bodies. If a single corpus amylaceum is examined in the schlieren grating microscope, it will appear as depicted in Figure 2. The microscopic field shows the background pattern, composed of straight parallel stripes, except where optical inhomogenities are present in the slide. There, characteristic displacements within the background pattern appear, clearly showing the contours of the specimen, in this case the corpus amylaceum. The accuracy of measuring and computing the refractive index, concentration, and dry mass can be increased if the specimens are mounted in several mounting media having different refractive indices. This has been done in the studies presented here. The calculations have been made in the following way: If n_C represents the refractive index of the corpus amylaceum, C the concentration of its mass, and M the dry mass, the following relations can be used:

$$n_C \sim n_M \cdot \left(\frac{D}{p}+1\right) \quad (1)$$

$$C \sim n_M \cdot \frac{D}{p} \cdot \frac{1}{\alpha} \quad (2)$$

$$M \sim n_M \cdot \frac{D}{p} \cdot \frac{1}{100 \cdot \alpha} \cdot \frac{4}{3} \cdot \pi \cdot r^3 \quad (3)$$

Here, n_M represents the refractive index of the mounting medium; D, the displacement of a line; p, the distance between the displaced portion of the line and the geometrical center of the corpus amylaceum; α, the specific refractive increment, and r, the radius of the corpus amylaceum under investigation. The specific refractive increment, α, is a constant depending upon the chemical composition of the object. For proteins, for instance, a value of 0.0019 is generally accepted. Other compounds of biological interest have the following values for the specific refractive increment, according to Davies, Wilkins, Chayen, and

* References 3-5.

LaCour [1]: Lipoproteins, having a 75% fat content, about 0.0017; nucleic acids, about 0.0020 or 0.0016 through 0.0017; carbohydrates and fats, about 0.0014, and salts, about 0.0012 through 0.0021. On the basis of the aforementioned histochemical investigations by Steele and co-workers and by Diezel, the main chemical component of the corpora amylacea may be considered to be mucopolysaccharides. Therefore, a value of 0.0014 for the specific refractive increment has been used in the calculations.

The specimens were mounted in water, glycerine, toluene, and carbon disulfide, and in Piccolyte (a commercial mounting medium). In all of those liquids, and also in formalin and ethanol, these bodies were found to be insoluble. The averages of the actual measurements, obtained on enlarged prints of photomicrographs, are listed in the Table. The refractive indices of all mounting media used, except that of carbon disulfide, are listed in the Table. As far as water and carbon disulfide are concerned, no accurate measurements could be done. In water, interfering edge effects occurred; also, in some of the measurements the displacement values became negative; i. e., the background pattern appeared to be convexly deformed, instead of showing the normal narrowing.

The Table, furthermore, contains the displacements, D, and the correlated distances, p, between the displaced lines and the centers of the corpora amylacea. The D/p ratio has been computed separately in each case, but, for simplicity, only the arithmetical averages are listed in the Table. The sixth column contains the values for n_C, the refractive index; the next, $C/100$ ml., the concentration, expressed in grams per 100 cc, and M, the mass of single corpora amylacea, expressed in grams. It should be kept in mind that this mass does not mean the actual total weight, but indicates the dry, or anhydrous, mass, partly depending upon the mounting medium. Hence, C and M could become zero in the case of mounting in Piccolyte.

The refractive index of single corpora amylacea lies in the range of $n_C = 1.43 \ldots 1.57$. Only one measurement (water) has been too low (1.25). As an average value of the refractive index, $n_C = 1.48$ was found. If the one value, being obviously too low, is excluded, the average would be $n_C = 1.51$. That figure is in agreement with the observation that these corpora amylacea if mounted in Piccolyte ($n_C = 1.531$) became practically invisible. For the mass C, expressed in grams per 100 ml. of solution, an average value of 52 gm. per 100 ml. was computed. In some of the corpora amylacea, however, a center is visible which appears

Mass Determinations on Corpora Amylacea from the Spinal Cord

Diameter of Corpora Amylacea, μ	Mounting Medium (with Refractive Index)	D	p	D/p (Average)	n_c	$C/100$ Ml. (Mass per 100 Cc. Solution)	M (Mass, gm.)
11.8	Water (1.333)	1.5 1.6	22 17	0.081	1.44	57	490×10^{-11}
15	Water	−1.7 −1.5	26 25	−0.063	1.25	--	--
--	Water	1.7	22	0.077	1.43	54	--
6.3	Glycerin (1.461)	1.0 1.0 0.7 0.9 1.0	16 12 10 12 17	0.070	1.56	54	70×10^{-11}
12.6	Glycerin	2.0 2.1 1.3	26 20 26	0.077	1.57	59	620×10^{-11}
7.9	Toluene (1.496)	0.9 0.8	18 19	0.046	1.56	36	93×10^{-11}
--	Piccolyte (1.531)	(0)		(0)	1.53	--	--

to be more dense than the periphery. The mass, M, of single corpora amylacea lies in the range between 70 and 620×10^{-12} gm., depending upon their diameters. The average mass for a single corpus amylaceum was computed to be 320×10^{-12} gm. This figure refers to an entire corpus amylaceum and not to the portion visible in a relatively thin histological section.

These values for the refractive index, mass concentration, and anhydrous mass indicate that these bodies represent relatively compact elements of a high degree of polymerization, provided their main chemical component are carbohydrates. That would be in good agreement with the histochemical findings. Since the results reported here are based only upon purely physical and optical analyses and since any chemical treatment or alteration of the examined object has been avoided, they confirm the findings obtained by histochemical methods and furnish certain quantitative data concerning these bodies.

Summary

Corpora amylacea from the spinal cord, analyzed by an optical schlieren method, showed an average refractive index of 1.51, a mass concentration of 52 gm. per 100 ml., and a dry mass of 320×10^{-12} gm.

Dr. O. J. Lowy supplied the histological material, and Mr. Gilford R. Millard took the photographs.

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Histochemical Studies of Lymph Nodes in Disseminated Lupus Erythematosus

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Most observers favor the hypothesis that the initial alteration of disseminated lupus erythematosus occurs in the deoxyribonucleic-acid metabolism of mesenchymal cells.* The historical background for this concept is based on the following observations and hypothesis: The L. E. cell was described by Hargraves, Richmond, and Morton.[4] This cell is thought to be the result of engulfment of altered nuclear material by the polymorphonuclear leukocyte. The discovery of this phenomenon led to the recognition of a constituent in the serum of patients with disseminated lupus erythematosus. This substance is apparently responsible for the production of the L. E. cell and is probably a protein in the γ-globulin fraction of serum.[5] It appears to have a selective effect on the nucleoprotein of the nucleus and is thought to be activated by a substance which is in part derived from platelets.† The effect of the γ-globulin substance is enhanced by previous cellular trauma.[3] The nucleoprotein released from the involved mesenchymal cell may be responsible for the changes, in the ground substance of the tissues, present in cases of disseminated lupus erythematosus.[6]

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* References 1-3.

† References 6, 7.

The counterpart in tissue of the L. E. cell is believed to be the hematoxylin body.[1] This structure has a variable content of nucleic acid, which is attributed to a gradual degradation and eventual disappearance of this component.‡ Complete loss of the nucleic acid would then account for the finding of eosinophilic "hematoxylin bodies."[2] These are presumed to contain the residual protein of the nucleoprotein and carbohydrate derived from altered ground substance.[1]

The histopathologic changes present in lymph nodes removed at operation and autopsy from patients with disseminated lupus erythematosus have been described previously.§ Using this same material as a basis for the present work, an amplification of the present concept of the pathogenesis of this disease will be presented. It is based on the histochemistry of the cytoplasm of plasma cells and of inclusions in the cytoplasm of some of these cells early in the clinical course of the disease.

Materials

Twenty-four cases of disseminated lupus erythematosus, observed at University Hospitals of Cleveland from 1945 to 1955, form the basis of the present report. In all, the clinical course of the patient's illness was consistent with this diagnosis. In 17 instances material obtained at autopsy was available for study. In seven of these cases, and in seven others in which autopsy had not been performed, lymph nodes, which had been removed at biopsy, were examined.

The lymph nodes from these cases had been fixed in 10% formalin.

‡ References 1, 9.

§ Moore, R. D.; Weisberger, A. S., and Bowerfind, E.: An Evaluation of Lymphadenopathy in Systemic Disease, A. M. A. Arch. Int. Med., to be published.

Methods

In all cases, sections from the lymph nodes were examined after the following procedures: Initially, a hematoxylin-eosin stain and a periodic acid-Schiff reaction[10] were done. Also, sections of tissue were treated prior to the PAS technique by acetylation and by acetylation followed by potassium hydroxide.‖ In addition, incubation in 1 N hydrochloric acid for 12 minutes at 60 C and for 3 hours at 37 C,[10] and incubation in solutions of pepsin, trypsin, ribonuclease, diastase, β-glucuronidase, and hyaluronidase were carried out on tissue sections before applying the PAS procedure.¶

A Feulgen reaction[35] was performed in all cases.

All tissue sections were stained with Sudan black.[12]

Representative material was treated with a 0.001% solution of toluidine blue for 10 minutes and examined with wet and dehydrated mounts.[12] This was done before and after exposure to pepsin and trypsin.

Sulfation of the tissue was carried out with a concentrated solution of sulfuric acid prior to staining with toluidine blue.[36]

A methyl green-pyronin stain was performed before and after treatment of the sections with ribonuclease and 1 N hydrochloric acid at 37 C for three hours.#

The Millon test[39] for protein was carried out before and after the use of trichloroacetic acid, trypsin, and pepsin.

Control sections from the same lymph nodes were examined in all instances.

Results

As was reported previously,* plasma cells were present in increased numbers in all lymph nodes from cases of disseminated lupus erythematosus. These cells were in higher concentration adjacent to and within sinusoids but were identified throughout the tissue. Occasional cells, interpreted as plasma cells, contained intracytoplasmic structures (Fig. 1). These inclusions were eosinophilic when stained with hematoxylin and eosin. They were present as single or multiple small granules, but occurred most frequently as a large mass which displaced the nucleus to the periphery of the cell (Figs. 2 and 3). These intracytoplasmic structures were present in all lymph nodes. Only a few per section was the usual find-

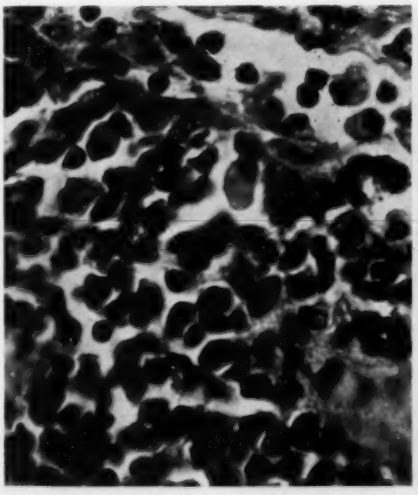


Fig. 1.—Lymph node obtained by biopsy which illustrates cells with multiple inclusions in the cytoplasm and displacement of the nucleus to the periphery of the cell. They are present in the pulp of the lymph node and in a sinusoid. Hematoxylin and eosin; × 600.

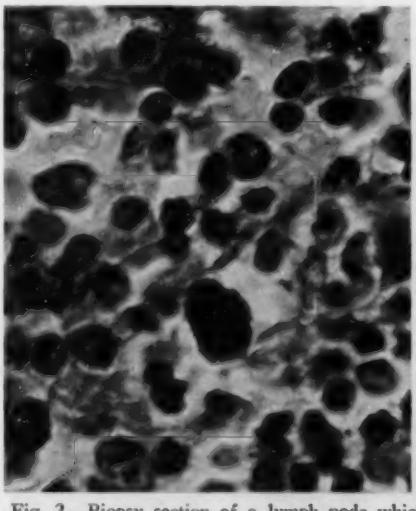


Fig. 2.—Biopsy section of a lymph node which illustrates typical cellular inclusion. Note the masses of nuclear material. Periodic acid-Schiff reaction; × 1000.

‖ References 11, 12.

¶ References 12, 14.

References 12, 13, 17.

* Moore, R. D.; Weisberger, A. S., and Bowerfind, E.: An Evaluation of Lymphadenopathy in Systemic Disease, A. M. A. Arch. Int. Med., to be published.

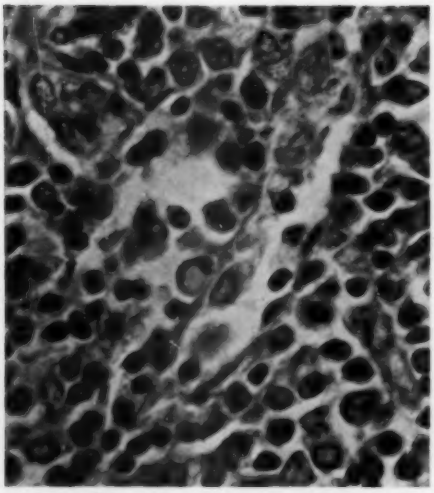


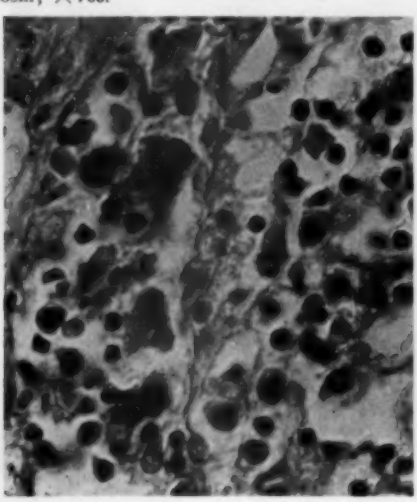
Fig. 3.—Intracytoplasmic inclusion which has displaced the nucleus to the periphery of the cell. The lymph node was obtained by biopsy and illustrates the negative Feulgen reaction of the inclusion. Feulgen reaction; × 600.

ing, but occasionally they were numerous.

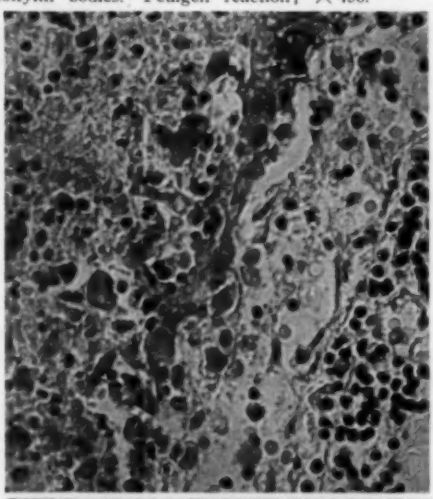
Results with the PAS reaction were similar to those reported in a previous paper.* The intracytoplasmic inclusions were strongly positive, and the reaction of the cytoplasm of the plasma cells varied from negative to intensely positive. The majority of these cells always had some reaction of the cytoplasm with the PAS technique.

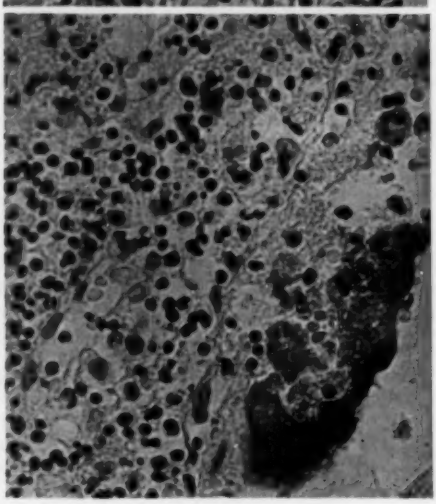
Hematoxylin bodies were identified in nine of the cases which were autopsied. They appeared to be a fusion of intracytoplasmic, PAS-positive structures which had been released from cells. Nuclear remnants were recognized in some of these bodies (Figs. 4, 5, and 6).

Fig. 4.—Lymph node obtained at autopsy which illustrates hematoxylin bodies and cells with intracytoplasmic inclusions. Note the apparent transition between the two structures. Hematoxylin and eosin; × 700.



Figs. 5 and 6.—Hematoxylin bodies in lymph nodes obtained at autopsy which show a positive Feulgen reaction. Note the more intense reacting clumps and bands of nuclear material in the hematoxylin bodies. Feulgen reaction; × 450.





The results of the various procedures on the cytoplasm of the plasma cells, intracytoplasmic inclusions, and hematoxylin bodies are presented in the accompanying Table. The reactions of the hematoxylin bodies are similar to those described by others.†

Results of Histochemical Procedures

	Hematoxylin Bodies	Plasma-Cell Cytoplasm	Intracytoplasmic Inclusions
PAS reaction	4+	0-4+	4+
Acetylation+PAS reaction	0	0	0
Acetylation+KOH+PAS reaction	4+	0-4+	4+
HCl (1 N at 60 C for 12 min.)+PAS	4+	0-4+	4+
HCl (1 N at 37 C for 60 min.)+PAS	4+	0-4+	4+
Pepsin+PAS reaction	4+	0-2+	4+
Trypsin+PAS reaction	4+	0-2+	4+
Ribonuclease+PAS reaction	4+	0-4+	4+
Diastase+PAS reaction	4+	0-4+	4+
β-glucuronidase+PAS	4+	0-2+	4+
Hyaluronidase+PAS	4+	0-4+	4+
Feulgen reaction	0-4+	0	0
Sudan black	0	0-1+	0
Toluidine blue (metachromasia)	0	0	0
Trypsin+toluidine blue (metachromasia)	0	0	0
Pepsin+toluidine blue (metachromasia)	0	0	0
H_2SO_4+toluidine blue (metachromasia)	0	0	0
Methyl green-pyronin (pyronophilia)	4+	0-4+	4+
HCl (1 N at 37 C for 3 hr.)+methyl green-pyronin (pyronophilia)	3+	0-2+	3+
Ribonuclease+methyl green-pyronin (pyronophilia)	3+	0-2+	3+
Millon reaction	4+	2+	4+
Trypsin+Millon reaction	0-1+	0	0-1+
Pepsin+Millon reaction	0-1+	0	0-1+

Comment

From the results of these procedures, certain inferences regarding the structural components of the cytoplasm of the plasma cells, cellular inclusions, and hematoxylin bodies may be made.

A positive PAS reaction which can be blocked by acetylation and reestablished with potassium hydroxide indicates a 1, 2 glycol linkage.‡ This suggests that a component within the cytoplasm of the plasma cells, the intracytoplasmic inclusions, and hematoxylin bodies is carbohydrate in nature. The more intense reaction in the intracellular inclusions and hematoxylin bodies indicates that they contain a high concentration of available reacting groups in the carbohydrate.

The degree of positivity of the PAS reaction in the cytoplasm of the plasma cells can be diminished by β-glucuronidase, pepsin, and trypsin. On the other hand, these enzymes did not induce changes in the cytoplasmic inclusions or the hematoxylin bodies. The decrease of the PAS reaction in the cytoplasm of the plasma cells in lymph nodes from cases of disseminated lupus erythematosus after incubation with β-glucuronidase suggests the presence of conjugated glucuronides.[21] If conjugated glucuronides are present in the cellular inclusions and hematoxylin bodies, the lack of change following incubation in β-glucuronidase indicates that they may not be in a form that the enzyme will act upon. The loss of intensity of the PAS reaction in the cytoplasm of the plasma cells after treatment with pepsin and trypsin could be explained as a loss of carbohydrate which had been linked in some manner to protein. Following enzymatic digestion of the protein, some of the carbohydrate would then be soluble in the solutions used in processing the tissue. The resistance of the inclusions and hematoxylin bodies to change in the PAS reaction after these enzymes would indicate that the released carbohydrate is no longer soluble. This, plus the lack of effect by β-glucuronidase, suggests a more complicated carbohydrate in the intracellular structures and hematoxylin bodies than in the cytoplasm of the plasma cells.

Failure of diastase to have an effect rules out the presence of glycogen as playing any part in the staining reaction.[14] Treatment

† References 1, 2.

‡ References 11, 19, 20.

with testicular hyaluronidase did not alter the results of the PAS technique. This would indicate that the carbohydrate is not hyaluronic acid or chondroitin sulfate.[12]

The PAS reaction in the cytoplasm of the plasma cells, cellular inclusions, and hematoxylin bodies was not changed by pretreatment of the tissue sections with hydrochloric acid or ribonuclease.§ This suggests that reacting groups had not been made available from ribonucleic acid.

The cytoplasm of the plasma cells, intracytoplasmic inclusions, and hematoxylin bodies were nonmetachromatic when stained with an aqueous solution of toluidine blue. Sulfation of the tissue prior to application of the dye failed to produce metachromasia.[16] Consequently, the presence of a long-chain polysaccharide with regularly arranged groups (that will react with a metachromatic dye) or with regularly arranged sites available for substitution is unlikely.[22] The possibility of masking of the reactive groups by protein was eliminated by digestion with pepsin and trypsin prior to staining.

There was no evidence of lipid with the Sudan black method except for occasional plasma cells with a few fine granules in the cellular and nuclear membranes.[23]

A positive pyronin reaction was present in the cytoplasm of the plasma cells, inclusions, and hematoxylin bodies. This pyronophilia was diminished, but not prevented, by pretreatment of the tissue with hot trichloroacetic acid, hydrochloric acid, and ribonuclease. These tests indicated that ribonucleic acid was present in these sites.‖

The Millon reaction was slightly positive in the cytoplasm of the plasma cells and strongly positive in the intracytoplasmic inclusions and hematoxylin bodies.¶ Pepsin and trypsin markedly decreased the positive reaction in all structures. It was concluded that a tyrosine-containing protein[18] was present in these sites, and in high concentration in the inclusions and hematoxylin bodies.

There was a negative Feulgen reaction of the cytoplasm of the plasma cells and the intracytoplasmic inclusions in all biopsy specimens of lymph nodes from patients with disseminated lupus erythematosus (Fig. 3). This finding ruled out the presence of deoxyribonucleic acid[15] in these sites at this stage of the disease. However, lymph nodes removed at autopsy disclosed many of the hematoxylin bodies to have a positive Feulgen reaction.# Also present in the lymph nodes removed at autopsy were rare cells with inclusions which contained a small amount of Feulgen-positive material.

When the histologic and histochemical alterations in lymph nodes obtained at biopsy from patients with disseminated lupus erythematosus are compared with lymph nodes obtained at autopsy, certain differences are apparent. These differences suggest possible modifications in the current concepts of the pathogenesis of this disease.

The initial change consists of a proliferation of all the cellular elements of the lymph node. The increase in plasma cells is marked. The metabolic activity of the plasma cells is increased, as manifest by the more intense reaction for ribonucleic acid in the cytoplasm.[17] Paralleling the increased intensity of the staining of ribonucleic acid, there is a positive periodic acid-Schiff reaction. This is the result of a greater concentration of carbohydrate within the cytoplasm of the plasma cells. In some of these cells, inclusions appear in the cytoplasm which have a similar, but consistently more intense, PAS reaction than the surrounding cytoplasm.

In addition, to the increased carbohydrate content, there is evidence of an increased concentration of protein in these inclusions.

The early changes observed in the lymph nodes cannot be explained by present concepts of the pathogenesis of disseminated lupus erythematosus. These place the alteration of deoxyribonucleic acid as primary,

§ References 12, 13, 17.
‖ References 12, 13, 17.
¶ References 1, 2.
# References 1, 2.

the accumulation of extracellular protein as a result of the breakdown of nucleoprotein, and the extracellular-carbohydrate increase as an effect of nucleoprotein on the ground substance.

Intact plasma cells with the intracytoplasmic inclusions were identified, adjacent to, or as a part of, the hematoxylin bodies (Figs. 4, 5, and 6). These latter structures were identified only in autopsy material. The plasma cells with inclusions were identical with those seen in the biopsy specimens.

Since extranuclear deoxyribonucleic acid is present only late in the course of the disease, it may be the result of nuclear breakdown from compression and distortion by the intracytoplasmic inclusions. However, necrosis of lymph nodes is also a terminal event, and is more prominent adjacent to the hematoxylin bodies. This suggests the possibility that the accumulation or release of the intracytoplasmic material into an extracellular location, followed by combination with extranuclear deoxyribonucleic acid may be responsible for the formation of the hematoxylin body.

Our observations indicate an accumulation of protein and carbohydrate within the cell early in the clinical course of the disease. Evidence of increased metabolic activity as manifest by increased amounts of ribonucleic acid suggests the elaboration of the protein-carbohydrate complex at this site. However, the possible phagocytosis of extracellular material cannot be excluded. The appearance of deoxyribonucleic acid in association with protein-carbohydrate complex to form the hematoxylin body occurs terminally. It is apparently the result of cell breakdown, release of intracytoplasmic inclusions, and adjacent tissue necrosis.

The changes we describe indicate altered cellular function. The plasma cells are present in increased numbers, and may be responsible for the elaboration or accumulation of excessive amounts of the protein-carbohydrate material that we have described. The increased number of metabolically active plasma cells will fit with the concept of an antigen-antibody etiology, as has been suggested by others.*

Several possibilities as to the relation of the changes in the lymph node to the systemic disease are suggested. The factor in the γ-globulin fraction of serum could be material released from the plasma cells. This substance, from the plasma cells, under the proper conditions, could cause the destruction of mesenchymal cells, with subsequent release and physical or chemical incorporation of nuclear products, to form the hematoxylin body. The precipitation of the protein-carbohydrate complex, with or without nucleic acid, could also account for its presence at other sites, such as the renal glomeruli. The inflammatory response in some organs and tissues may also be the result of accumulation of this (protein-carbohydrate) substance and the breakdown of cells. The L. E. phenomenon suggests a much speeded-up process of cell destruction, with phagocytosis of the cytoplasmic and nuclear debris by the polymorphonuclear leukocytes.

Summary

Hyperplasia of superficial lymph nodes occurs in disseminated lupus erythematosus. There are numerous plasma cells present which contain increased amounts of ribonucleic acid, protein, and carbohydrate within their cytoplasm. Some of the plasma cells develop intracytoplasmic inclusions, which give a characteristic configuration to the cell. These formed elements contain polysaccharide, tyrosine-rich protein, and ribonucleic acid. The release of the intracytoplasmic inclusions during the breakdown of the cells which contain them and the necrosis of the adjacent tissue are a terminal event in the lypmh nodes. At this time deoxyribonucleic acid is observed as a component of these structures, which appear as characteristic hematoxylin bodies.

The possible significance of these observations in the pathogenesis of disseminated lupus erythematosus is discussed.

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Coccidioidomycotic Thoracic Lesions in Dogs in Tucson, Arizona

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In the endemic area of coccidioidomycosis, man is not the only creature that becomes infected with Coccidioides immitis. The disease occurs spontaneously in a variety of animals. The thoracic lesions have been studied in cattle and in wild rodents.* Several cases of coccidioidomycosis have been reported in dogs.[9] In one case multiple small nodules, some necrotic and containing numerous cells of Coccidioides, were scattered through the lungs, liver, spleen, and kidneys.[4] In a second case[10] there were widely scattered hepatic lesions, with a maximum diameter of 1.5 cm., three similar nodules in the lung, and one in the spleen. Infection in dogs, however, must be much commoner than is revealed by the literature. Fuller,† in Tucson, studied three fatal cases. Therefore, a systematic search for arrested, or at least subclinical, lesions comparable to those of Ashburn and Emmons‡ in wild rodents and to ours[11] in human patients seemed appropriate.

Material and Methods

During February and March, 1955, in Tucson, Ariz., the lungs and tracheobronchial lymph nodes of 34 dogs were examined for possible lesions of coccidioidomycosis. The animals were killed in the Pima Animal Shelter. One was killed because of suspicion of distemper; the others were apparently healthy stray animals. Therefore, no particulars about age were available. No skin tests were done. All gross thoracic lesions found, as well as the smallest ones, were examined microscopically with hematoxylin and eosin, the Van Gieson stain for elastin, and the Gridley stain. No cultures were made.

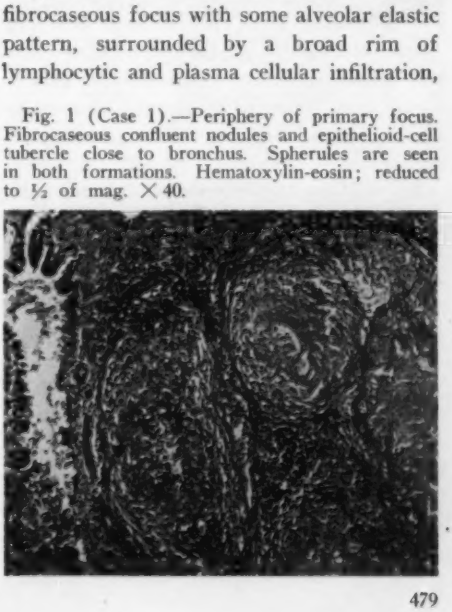
Tucson is in the endemic area of coccidioidomycosis, and a high incidence of the, mostly asymptomatic, disease has been established clinically, at autopsy, and by skin testing in man.

Results

In eight dogs lesions with organisms morphologically identical with C. immitis were found.

Case 1 (D 3).—One small firm grayish-yellow subpleural lesion, measuring 3 by 2 mm., was found. It consisted of a central fibrocaseous focus with some alveolar elastic pattern, surrounded by a broad rim of lymphocytic and plasma cellular infiltration,

Fig. 1 (Case 1).—Periphery of primary focus. Fibrocaseous confluent nodules and epithelioid-cell tubercle close to bronchus. Spherules are seen in both formations. Hematoxylin-eosin; reduced to ½ of mag. × 40.



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From the Clinical Laboratories, Jewish Hospital, and the Laboratory of Mycology, Cincinnati General Hospital (Departments of Pathology and Dermatology).

* References 1, 3, 7.

† Fuller, R. H.: Personal communication to the authors.

‡ References 1, 3.

in which small interstitial epithelioid granulomas were present (Fig. 1). In the caseous center many spherules were found. In the granulomas few spherules and some ruptured capsules were present. One of these granulomas was in the immediate vicinity of a small bronchus, but had not broken through to the lumen. The lymph nodes were without change.

Case 2 (D 5).—Three very small grayish subpleural lesions, measuring 1 to 2 mm. in diameter, were found. Two of these seemed to be nonspecific inflammatory lesions of anthracosilicotic nature. The third was a specific inflammatory lesion of the bronchial mucosa and wall and the surrounding interstitium. The infiltrating cells were lymphocytes and plasma cells. In the affected area a few scattered granulomas were found. They were composed mainly of epithelioid cells, and some showed slight caseation; one was fibrocaseous. No alveolar elastic pattern was discernible. Only two organisms were found on serial sections. One of these showed endosporulation (Fig. 2). The fibrocaseous lesion had broken through into the bronchial lumen (Fig. 3). The contributory, larger bronchus contained a mass of epithelioid cells, presumably resulting from the rupture of the main lesion into the bronchial lumen. Two isolated granulomas were found in the immediate vicinity of the main lesion. They were also situated in the bronchial wall. The lymph nodes were without change.

Case 3 (D 11).—In the left and in the right apical lobe one yellowish-gray, firm subpleural focus was found. The two lesions measured 2.5 and 5 mm. in diameter, respectively. Microscopically, they consisted of calcifying caseous material with some alveolar elastic pattern. They had a narrow fibrotic capsule with some lymphocytic infiltration and an occasional small epithelioid-cell granuloma (Fig. 4). In the caseous material were typical spherules in small numbers. The tracheobronchial lymph nodes were not enlarged. In a lymph node with a spherule in the center of one nodule four small epithelioid and caseating granulomas were found (Fig. 5).

Case 4 (TU 4).—In the left apical lobe and in the right diaphragmatic lobe a subpleural focus 2 mm. in diameter was found. The lesions consisted of a yellowish-gray center and a narrow gray hyaline capsule. Microscopically, the two foci showed an identical structure, comparable to the lesion in Case 1. The center was formed by fibrocaseous material, with some recognizable alveolar elastic pattern, some liquefaction, and very slight incipient calcification. The periphery consisted of a zone of lymphocytic-plasma cellular infiltration, with small interstitial epithelioid granulomas. The

Fig. 2 (Case 2).—Spherule of C. immitis with endosporulation in satellite lesion from lung. Gridley stain; × 1400.

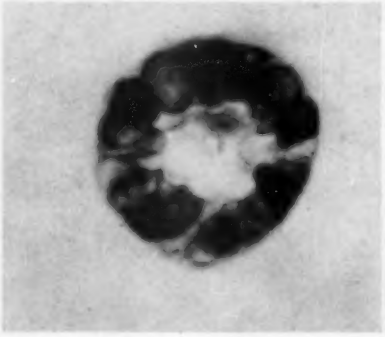


Fig. 3 (Case 2).—Rupture of coccidioidomycotic fibro-caseous granuloma into bronchial lumen. Hematoxylin-azophloxine; reduced to ½ of mag. × 220.

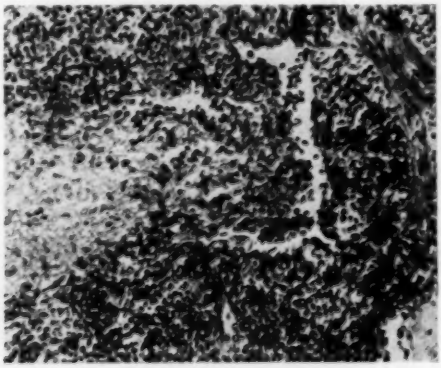


Fig. 4 (Case 3).—Discrete nodule with diffuse caseation and scanty calcification; primary focus. Notice small satellite lesions on the external border of the capsule. Hematoxylin-eosin; reduced to ¾ of mag. × 30.

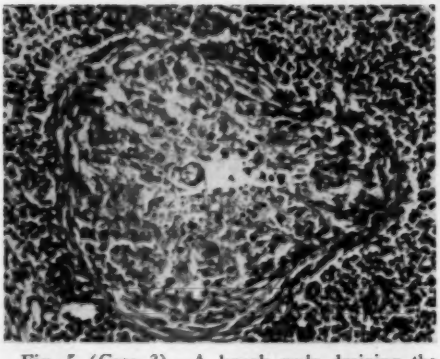


Fig. 5 (Case 3).—A lymph node draining the area of the caseous primary focus shows an epithelioid-cell tubercle with a central spherule of C. immitis. Hematoxylin-eosin; reduced to 53% of mag. × 230.

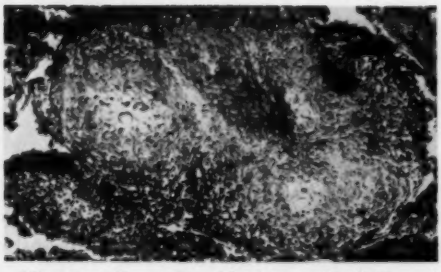


Fig. 6 (Case 4).—Multiple epithelioid cell tubercles in a lymph node of the primary complex. Beginning central caseation. Each nodule shows a central spherule. Hematoxylin-azophloxine; reduced to 53% of mag. × 110.

caseous center reached the pleura, and the granulomatous periphery entered wedge-shaped deep into the pulmonary tissue. In the caseous center and in the granulomas of the periphery typical spherules were present.

The lymph nodes were not enlarged. One had in a slightly caseating granuloma some spherules (Fig. 6).

Case 5 (D 4).—In a cardiac lobe near the hilus a firm subpleural lesion, 2 mm. in diameter, was found. The lesion consisted of slightly calcifying, dry caseous material with fissures of cholesterol crystals. The caseous lesion had a narrow fibrotic capsule with some lymphocytic infiltration. In this capsule and in the vicinity of the lesion small interstitial epithelioid granulomas could be visualized. In the caseous material and in these granulomas spherules were present (Fig. 7).

One lymph node contained a small caseating granuloma with some spherules.

Case 6 (D 12).—Around the right main bronchus, and fixed to it, a gray hyaline, somewhat fibrous "tumor," measuring approximately 3 to 4 cm. in diameter, was

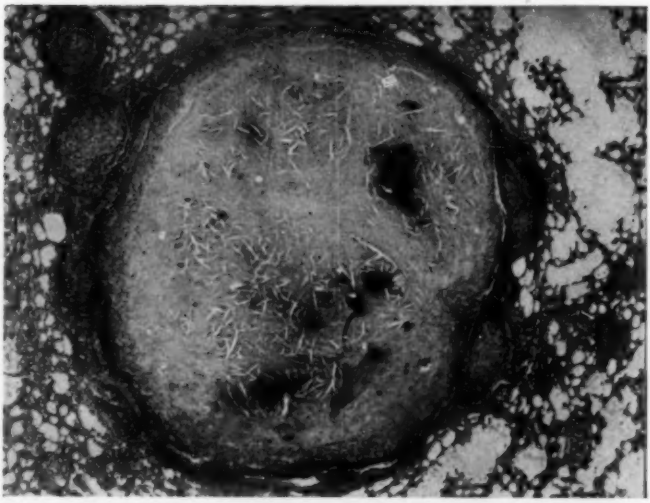


Fig. 7 (Case 5).—Well-circumscribed primary focus with extensive caseation, numerous cholesterol clefts, and beginning calcification. Notice the satellite granulomas outside the capsule. Hematoxylin-eosin; reduced to 80% of mag. × 30.

found. In the pulmonary tissue near the "tumor" was a subpleural gray focus 2 to 3 mm. in diameter. Between the "tumor" and the pulmonary lesion a hyperplastic lymph node 7 mm. in diameter was present. The "tumor" originated from the tracheobronchial lymph nodes and involved the bronchial wall. The pulmonary lesion consisted of a group of small granulomas amid rather dense lymphocytic-plasma cellular infiltration. Some of the granulomas were caseated and had a recognizable alveolar pattern. In the granulomas spherules were present. The lymph node "tumor" was almost completely fibrotic (Fig. 8). In the meshes of this fibrotic hyperplasia granulomatous tissue was found, consisting of lymphocytes, plasma cells, polymorphonuclear leucocytes, and histiocytes with a few scattered spherules. Small foci of caesation were found with a few giant cells and many spherules (Fig. 9). The indurated lymph

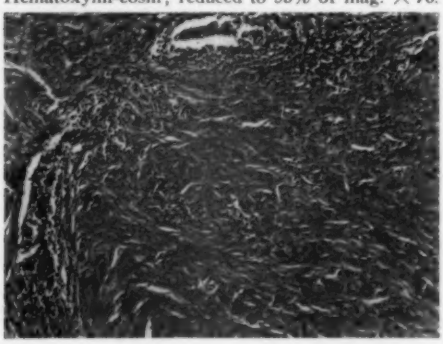


Fig. 8 (Case 6).—Fibrous and hyaline scar tissue following coccidioidomycotic lymphadenitis. Hematoxylin-eosin; reduced to 53% of mag. × 70.

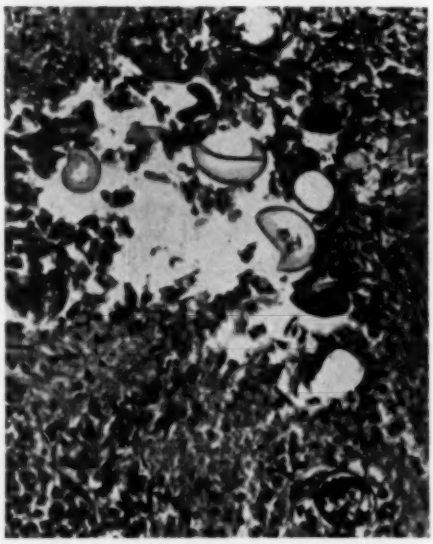


Fig. 9 (Case 6).—Necrosis and inflammation with numerous spherules in the vicinity of the scar. Notice round spherule with endospores and the irregular shells of the ruptured (empty) spherules. Hematoxylin-eosin; reduced to 53% of mag. × 200.

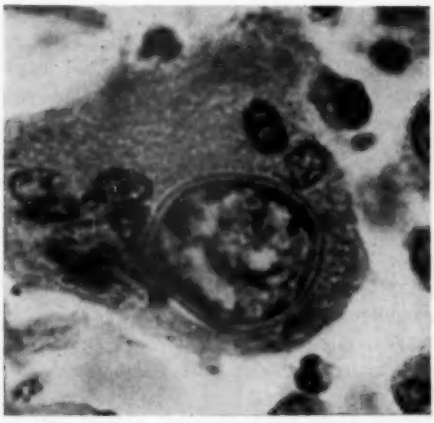


Fig. 10 (Case 7).—Intra-alveolar multinucleated giant cell with an endospore-containing spherule. Hematoxylin-eosin; reduced to 51% of mag. × 950.

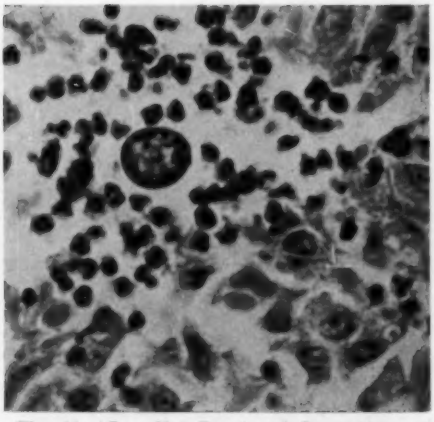
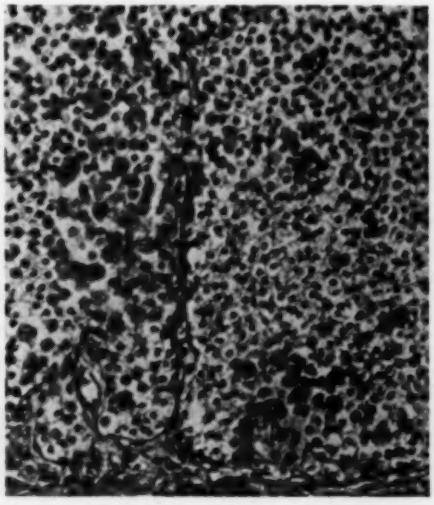


Fig. 11 (Case 7).—Purulent inflammation and epithelioid-cell proliferation in a lymph node of the primary complex with a spherule surrounded by polymorphonuclear leucocytes. Hematoxylin-eosin; reduced to 51% of mag. × 510.

node tumor involved the wall of a main bronchus. Here the bronchial mucosa showed monocytic infiltration; the bronchial epithelium was flattened. The hyperplastic lymph node had no specific changes but was distinctly silicotic.

Case 7 (D 6).—In both lungs extensive segmental consolidation without any pleuritis was found. The left paratracheal lymph nodes were swollen and formed a tumor-like mass measuring 4×3×2 cm. On incision this tumor showed hyalin fibrotic tissue with grayish-yellow mucopurulent suppuration. In most parts of the lung the microscopic picture was that of a severe acute bronchopneumonia without organisms. The exudate in bronchi and alveoli consisted of polymorphonuclear cells and many monocytes. It contained hardly any fibrin. In one bronchial wall destructive suppuration without organisms was found. In the adjacent peribronchial tissue there were a few granulomas with spherules. In one lobe the picture was somewhat different. Generally speaking, the same exudative bronchopneumonia was present. However, many alveoli contained large epithelioid cells, leading to the formation of intra-alveolar granulomas with a few giant cells. In these granulomas spherules in small number were found, occurring exceptionally in giant cells (Fig. 10). The lymph node "tumor" resembled that in Case 6. The granulomatous reaction was more pronounced. In the granulomatous sites between the areas of fibrosis were many polymorphonuclear cells, and even small abscesses with a few spherules were present (Fig. 11).

Fig. 12 (Case 8).—Marginal pneumonitis with polymorphonuclear- and round-cell exudate. Note the absence of pleuritic reaction and "specific" character of the inflammation. Hematoxylin-eosin; reduced to 51% of mag. × 175.



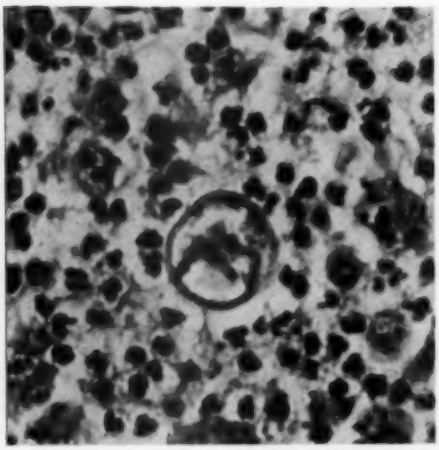


Fig. 13 (Case 8).—Regional suppurating lymph nodes, one of which contains this typical thick-walled spherule. Hematoxylin-eosin; reduced to 51% of mag. × 510.

Case 8 (D. D).—This dog was suspected of having distemper. Like Case 7, we found in both lungs extensive segmental consolidation without any pleuritis. The tracheobronchial lymph nodes were swollen and reached a diameter of 1.2 cm. Microscopically, the lung, again, showed severe bronchopneumonia with an abundance of polymorphonuclear cells mixed with many monocytes. In some bronchi the pus contained giant cells also. There was no intra-alveolar granuloma formation, and no spherules were found (Fig. 12). The results of the Grocott stain will be mentioned in the discussion. The lymph nodes were slightly indurated, with granulomatous fields consisting of polymorphonuclear cells, lymphocytes, plasma cells, and monocytes. In this granulomatous tissue were very few typical spherules (Fig. 13). One swollen lymph node without fibrosis showed similar granulomatous tissue without polymorphonuclear cells but with scattered giant cells, similar to Sternberg cells in Hodgkin's disease (Fig. 14). Elsewhere in the same lymph node an indication existed of the formation of small epithelioid granulomas. No spherules were present. In the cortical sinuses were many polymorphonuclear leucocytes.

Comment

As could be expected after our findings in autopsies on human patients in Tucson, Ariz.,[11] the yield of positive cases, i. e., cases with lesions in which morphologically typical organisms were present, in this small series of dogs was considerable. In the 34 dogs examined we found 8 positive cases, a figure comparable to the 8 positive cases

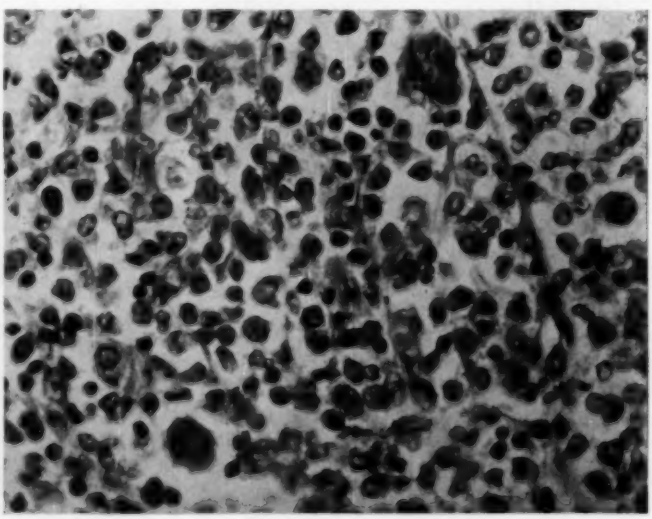


Fig. 14 (Case 8).—Morphologic picture simulating Hodgkin's granuloma in a lymph node of a primary complex. Multinucleated giant cells. Hematoxylin-eosin; reduced to 75% of mag. × 510.

which we found in 40 patients dying of causes other than coccidioidomycosis. Like the patients, the dogs formed an unselected series. Only in one case was the animal killed because of disease; the others were stray animals and were regarded as healthy.

In four of our cases (Cases 1, 3, 4, and 5) the lesions were comparable to those in man. They showed small subpleural pulmonary lesions which were arrested or undergoing arrest and could be defined as primary foci. The center of the lesion was a small caseating focus with recognizable alveolar pulmonary elastic pattern, measuring from 1 to 5 mm. in diameter. In two dogs (Cases 3 and 5) this caseous material showed beginning calcification. Formation of bone was not encountered. The capsule of these pulmonary foci was more granulomatous than in our cases from human material. The findings in dogs give support to our conclusion, based on the human material, that the capsule of the primary coccidioidal focus was the result of interstitial granulomatous extension of the disease process.

In contradistinction to our cases in man, we found a double primary focus in two instances (Cases 3 and 4). The structure of the two foci in both dogs was identical. They must be regarded as the result not of consecutive, but of simultaneous, injections.

The primary focus in Case 2 was different from what we have seen in the four cases mentioned or in man. It was situated completely in the bronchial wall. Even in serial sections no involvement of the pulmonary tissue was found. Neither in histoplasmosis nor in tuberculosis have we ever seen such a localization. This case was also remarkable in other respects. The lesion in the bronchial wall had broken through into the bronchial lumen. In the tributary bronchus part of the content of the bronchial lesion was recognizable. We feel that in this case we caught a bronchogenic dissemination in the making. A primary localization of coccidioidomycosis in the bronchial wall may be of importance for dissemination along the bronchial tree.

In three of these five cases of localized pulmonary coccidioidal infection (Cases 3, 4, and 5) a very discrete dissemination to the tracheobronchial lymph nodes was found. In these three cases we are allowed to speak of a primary complex, albeit an abortive one. In dogs, as in man, subclinical infection with C. immitis seems to cause a much less pronounced primary complex than is the case in histoplasmosis, or even in tuberculosis.

In marked contradistinction to this conclusion are the findings in Cases 6, 7, and 8. Here tumor-like major involvement of lymph nodes was present. We feel, however, that in these cases it is not correct to speak of arrested lesions, or even of lesions which are undergoing arrest. The lymphadenitis showed some induration as a sign of chronicity, but the suppurative reaction was evidence of activity. These cases are examples of beginning disseminating disease, such as we did not encounter in man. They give support to our conclusion, based on the human material, that in coccidioidomycosis spread to the lymph nodes is already a disseminating form of the disease, and no longer a subclinical manifestation. In one of these three cases (Case 6) the lymph node disease could be ascribed to dissemination from a small pulmonary lesion that was not different from those found in Cases 1, 3, 4, and 5. In Cases 7 and 8, however, an extensive pulmonary inflammation was present that originally did not give rise to a suspicion of coccidioidomycosis. The same can be said of the microscopic picture. It was mainly that of an acute bronchopneumonia. No spherules were found. At first the process was regarded as secondary to bronchial obstruction by the tumor-like coccidioidomycotic involvement of the hilar lymph nodes. In that case, however, it was remarkable that with the Gram stain no micro-organisms could be found and that

the pleura immediately adjacent to the pneumonic process did not show the slightest sign of reaction. Remarkable also was the fact that at least in Case 7, in one of the pneumonic segments, grossly not different from the others, the acute process was complicated by intra-alveolar and interstitial granulomatous inflammation in which spherules could be detected. Therefore a coccidioidomycotic etiology of the whole pneumonic process had to be considered.

The work of Forbus [5] is the only report on the histologic characterization of pulmonary coccidioidomycosis. He stated that the histologic picture of primary coccidioidomycotic pneumonia is not at all specific. The pulmonary exudate consists largely of polymorphonuclear cells with a scattering of large macronuclear wandering cells and a considerable amount of fluid without fibrin. The lesions relate in particular to the terminal portions of the bronchial system and the immediately adjacent atria and alveolar spaces. The bronchi are usually filled with exudate, consisting of leucocytes and fibrin. Necrosis, abscess formation, and granulomatous transformation may result. The picture is characterized only by the presence of the organisms. The reaction to the spherules is mononuclear; that to the endospores is polymorphonuclear. The reaction to the arthrospores is known only experimentally. According to Cronkite and Lack,[2] it is granulomatous, and not polymorphonuclear. Guinea pigs showed already eight days after inhalation of arthrospores multiple small granulomatous lesions with spherules. These small lesions must be regarded as primary foci, which in later stages lead to fibrosis.

Forbus'[5] description of primary pulmonary coccidioidomycosis is completely valid for the lesions found in Case 7, except that in those parts of the pneumonic process without granulomatous changes at first no specific organisms could be detected, even with the Gridley stain. Endospores are even more difficult to find with the hematoxylin-eosin stain. The Gridley stain also has its

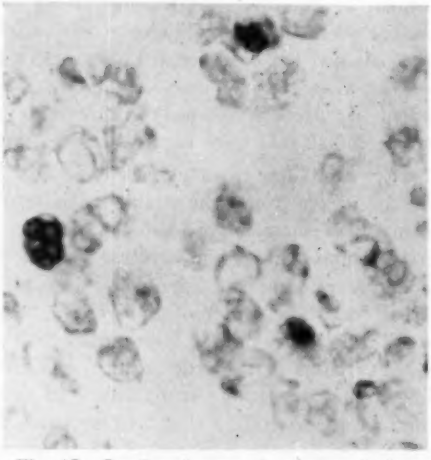
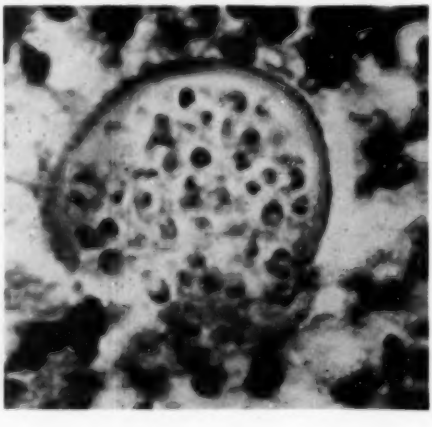


Fig. 15.—Small endospores in clusters in bronchial exudate. Gridley stain; reduced to 51% of mag. × 950.

limitations. By use of the Gridley stain in a case from Dr. Fuller's postmortem material, we found organisms in the bronchial exudate smaller than any in our own material (Fig. 15). Some empty shells pointed to their coccidioidomycotic nature. If these small organisms, often arranged in clusters, were endospores, they still were larger than the definite endospores shown in Figure 16 in a ruptured spherule. Even such small organisms were absent in the nongranulo-

Fig. 16.—Ruptured spherule of C. immitis emptying endospores into the tissue. Case of Dr. Fuller. Hematoxylin-eosin; reduced to 59% of mag. × 1400.



matous parts of the pneumonic process in Cases 7 and 8 stained with the Gridley stain. Therefore, we tried the recently published Grocott [6] silver stain. This is indeed an ideal fungus stain. It gives no precipitate, and elastic tissue and the bronchial mucus take the silver and make possible an easy orientation in the pulmonary tissue. We used *Kernechtrot* as a counterstain. With the Grocott stain in the pneumonias in Cases 7 and 8 small black rings were made visible in the macronuclear wandering cells of the exudate (Fig. 17). In size and

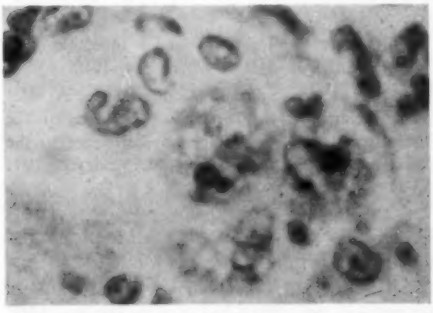


Fig. 17 (Case 7).—Endospores in a large alveolar cell. Endospores can be reliably diagnosed only in the presence of spherules or by culture. Grocott stain; reduced to 59% of mag. × 1400.

shape these rings are comparable to the endospores in Figure 16. Of course, cultural proof is lacking, but morphological evidence favors their being endospores of Coccidioides.

As stated above in our material in dogs (Fig. 2) and in the arrested lesions in man, endospores as a rule were much larger than those in Figure 16. Emmons and Ashburn [3] were impressed by the few endosporulating organisms they found in the lesions in wild rodents as compared with progressive human lesions. They regarded suppression of maturation and of endosporulation as a result of host influence. We are struck by the large size of the endospores in arrested lesions. This may be a result of host influence also. It has to be considered that in the more chronic granulomatous changes the endosporulation which we find is of the giant type (Schenken [8]) and that only in more acute cases are the regular forms seen. In that case, the difference between exudative and productive coccidioidomycotic lesions would be in part due to "allergy" of the organism.

On the evidence of the histologic picture, Forbus [5] came to the conclusion that primary pulmonary coccidioidomycosis may resolve completely without residual structural changes as long as no necrosis, abscess formation, or granulomatous transformation has occurred. Clinically, such lesions in primary pulmonary coccidioidomycosis are well known. Pleuritis may be absent even with extensive pulmonary consolidation. It is only the granulomatous primary focus (Cronkite and Lack [2]) or the granulomatous reaction to the dissemination of mature and giant spherules that leads to induration and caseation and to the arrested lesions that remain recognizable by the presence of large spherules. It is quite conceivable that pneumonia resulting from early bronchogenic spread of small endospores originating from the organisms in the primary focus is prone to complete resolution. In such cases a careful search for a localized suppurative lesion, possibly in the bronchial wall or in a lymph node, may give a clue to the pathogenesis. Such a meticulous search was not done in our Cases 7 and 8. In Case 7, however, we found an abscess in the bronchial wall with a few coccidioidomycotic granulomas in the adjacent peribronchial tissue. In both cases major suppurative lesions in the tracheobronchial nodes were seen.

The acute pneumonia in these cases may result in complete resolution if it is caused by endospores, as the small organisms found with the Grocott stain suggest. After eventual resolution, the primary focus, the source of the endospores, will remain. It still may grow and, as a reaction to the giant forms of the organisms, may give rise to a localized lesion with the character of a "coccidioma."

We are well aware that we have only circumstantial evidence for the reconstruc-

tion of the pathogenesis of coccidioidomycosis. The same holds true for the specific nature of the granulomatous reaction resembling Hodgkin's disease in one lymph node in Case 7. Our investigation has shown that a systematic cultural and morphological examination in dogs may be helpful in elucidating some questionable points in the pathogenesis of primary coccidioidomycosis.

Summary

In a series of 34 dogs coccidioidomycotic lesions were found in the lungs or in the hilar lymph nodes in 8 cases.

All dogs except one were stray animals without distinct clinical disease.

In two dogs a small pulmonary primary focus was seen, and in two others an abortive primary complex was found. In one dog the primary focus was situated in the bronchial wall and had broken through to the bronchial lumen. Twice a double primary focus was encountered; in two cases the primary foci showed some calcification.

In three dogs there was extensive, partly suppurative involvement of the hilar lymph nodes. In one of these the lymph node disease could be regarded as a dissemination from a small pulmonary primary focus; in the two others there was extensive acute bronchopneumonia. This pneumonia was partly granulomatous, with typical organisms, and partly nongranulomatous, apparently without typical organisms. Only with the Grocott stain could small structures resembling endospores be found.

This project was made possible by the use of the facilities of the laboratory of St. Mary's Hospital, Tuscon, Ariz.

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Necrotizing and Healing Pulmonary Arteritis with Advanced Mitral Stenosis

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In 1926 Von Glahn and Pappenheimer [1] described a form of arteritis in the lungs and in isolated systemic vessels in cases of rheumatic heart disease. This lesion was considered to be specific for rheumatic fever. Both acute and healed forms were described. Parker and Weiss,[2] in their studies on anatomic alterations of the lung in the presence of mitral stenosis, described five instances of necrotizing pulmonary angiitis. Larrabee, Parker, and Edwards [3] studied the intrapulmonary arterioles in 20 selected cases of advanced mitral stenosis. They noted that a few pulmonary vessels had occlusive lesions. These lesions morphologically resembled organizing thrombi, while other vessels revealed scars interpreted as a healed consequence of dissecting aneurysm or an inflammatory process. These lesions resembled the healed lesions originally described by Von Glahn and Pappenheimer. Recently Braunstein [4] reported six cases of pulmonary arteritis that resembled polyarteritis nodosa. Three of these cases had mitral stenosis and two had advanced pulmonary emphysema. He suggested that the common factor responsible for the development of the arteritis in these cases was pulmonary hypertension. In an experimental study on animals, Ferguson [5] produced pulmonary vascular lesions by the anastomosis of a systemic artery to a pulmonary artery. After removal of the anastomosis the pulmonary arteritis healed with scarring, and lesions appeared to be similar to those described by Von Glahn and Pappenheimer. In view of these observations and the fact that somewhat similar forms of necrotizing angiitis may occur in certain types of congenital heart disease, it was decided to re-evaluate pulmonary arteritis associated with rheumatic fever.

In the autopsy series at Presbyterian Hospital, 26 cases with this form of pulmonary arteritis in patients with rheumatic heart disease were studied. These vascular lesions included both acute and healed forms. Some of the earlier cases were those originally reported by Von Glahn and Pappenheimer. The records of the 26 cases were analyzed for clinical and anatomic evidence relating to activity of rheumatic fever. The anatomic findings regarded as stigmata of possible rheumatic fever activity were Aschoff nodules, verrucous endocarditis, active auricular endocarditis, and rheumatic pneumonia. Clinical evidence included such findings as fever, increased erythrocyte sedimentation rate, joint manifestations, chorea, and skin nodules. The type and degree of heart-valve alteration were evaluated. Particular attention was accorded to evidence of pulmonary thromboembolism. The arteritis was examined to determine whether there were any features that might distinguish it from other forms of arteritis (hypersensitivity, granulomatous, and periarteritis). The cases with isolated systemic arterial lesions were similarly studied.

Table 1 relates in particular the degree and type of valve involvement; the incidence of rheumatic activity, either clinical or anatomic, and the presence of systemic arteritis with the presence of pulmonary arteritis. Table 2 indicates the relation be-

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TABLE 1.—*Relation Between Pulmonary Arteritis and Mitral Stenosis*

No. of Cases of Pulmonary arteritis	Male	Female	No. of Cases with Advanced Mitral Stenosis	No. of Cases with Systemic Arteritis	No. of Cases with Evidence of Rheumatic Activity
26	9	17	26	1	12

TABLE 2.—*Relation Between Pulmonary Arteritis and Evidence of Activity of Rheumatic Fever*

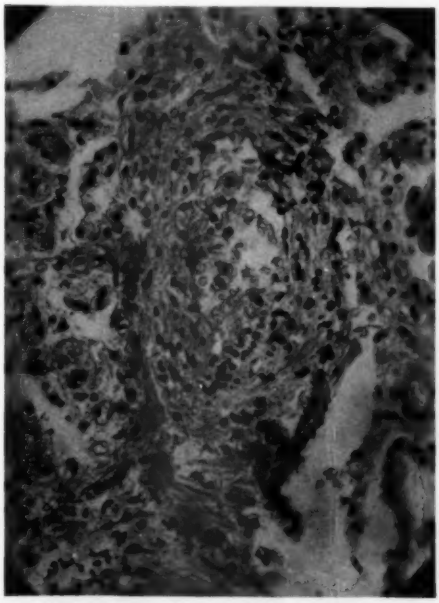
Character of Vascular Lesions	No. of Cases	No. of Cases with Evidence of Rheumatic Activity
Acute pulmonary arteritis	10	5
Acute and healed pulmonary arteritis	5	2
Healed pulmonary arteritis	11	5
Total	26	12

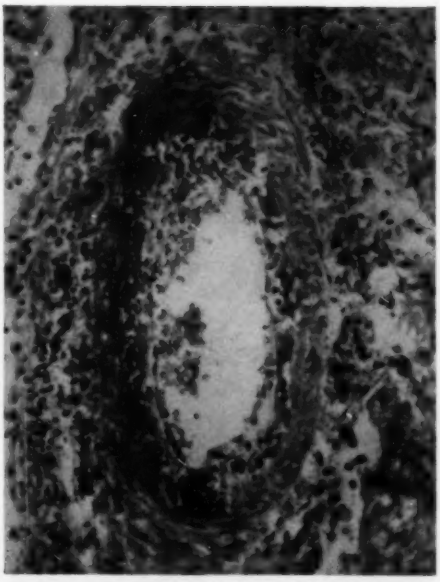
tween the type of pulmonary vascular lesion and evidence of rheumatic activity.

It may be noted that the significant valve alteration present in every case was an advanced mitral stenosis. In an analysis of autopsies in over 600 cases with rheumatic heart disease, pulmonary arteritis, acute or healed, was never present in the absence of an advanced mitral stenosis. There was also no correlation of the acute or healing phase, or lack of activity of the pulmonary vascular lesion, with the activity of the rheumatic process. Half the cases with acute pulmonary arteritis were unassociated with Aschoff nodules, verrucous endocarditis, rheumatic pneumonia, or auricular endocarditis, or with clinical manifestations of acute rheumatic fever. However, half the healed pulmonary vascular lesions were associated with such findings.

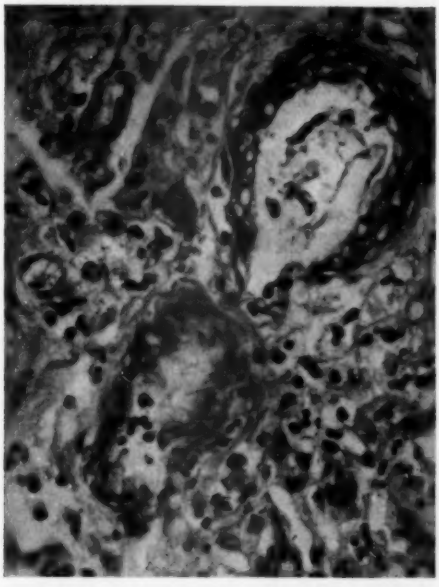
In 10 of the 26 cases there was evidence of pulmonary thromboembolism. Only 1 of the 26 patients had systemic arteritis. This was limited to a single vessel in the renal pelvis. Because of this case, and because Von Glahn and Pappenheimer [1] described a somewhat similar form of arteritis in rheumatic fever in sites other than the lung, all cases with extrapulmonary arteritis in rheumatic heart disease were reviewed. There were 15 such cases, including the one cited in association with the pulmonary vascular lesions. These 15 cases revealed involvement of coronary, testicular, and renal arteries. Differences existed in certain respects between some of the findings in cases with extrapulmonary arteritis and those in cases with pulmonary arteritis. In the cases with systemic involvement, at most only one or two vessels were found to be altered, as compared with the pulmonary form, in which many vessels were implicated. Of perhaps greater significance is the fact that in all 15 instances with systemic arteritis definite evidence of acute active rheumatic fever was present. Obviously, the occurrence of the systemic lesions was not necessarily associated with an advanced degree of mitral stenosis. The systemic vessels involved appeared to be larger than those in the lungs, and healed systemic arteritis was not seen.

The active pulmonary lesions involved the smaller and medium-sized vessels, particularly at points of bifurcation. The entire thickness of the wall was usually involved, although not always uniformly around the entire circumference of the vessel. Necrosis was fibrinoid or eosinophilic in type and was associated with an intramural and a perivascular infiltration with a pleomorphic group of inflammatory cells (Figs. 1, 2, and 3). Eosinophilic leucocytes were not a





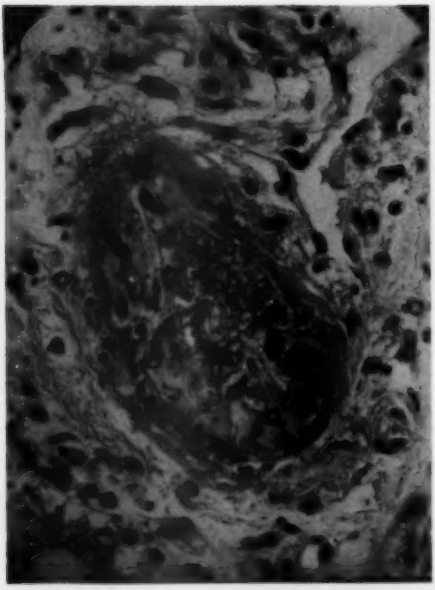
Figs. 1, 2, 3.—Photomicrographs of pulmonary vessels showing inflammatory infiltration and fibrinoid necrosis of walls; hematoxylin and eosin.

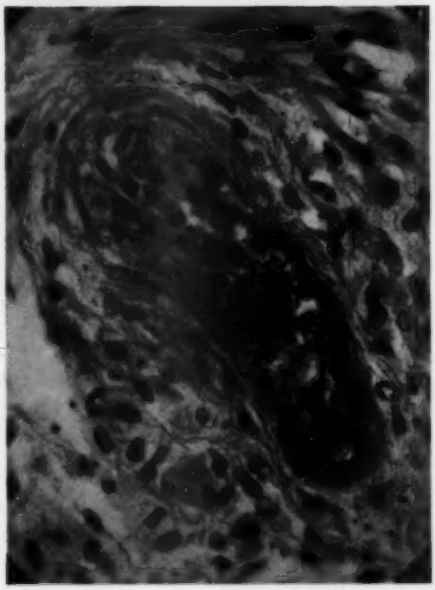


prominent feature. Giant cells were not observed. Red blood cells were frequently extravasated into the wall. Necrosis involved the cellular constituents of the wall, as well as the elastica at times. The healed lesions consisted of focal scars with new vascular formation in the walls, along with marked intimal fibrous proliferation. This often obliterated the entire lumen, and at times recanalization was present (Figs. 4, 5, and 6). It is the latter lesion that could not with certainty be distinguished from healed thrombosis. No perivascular inflammatory infiltrate was present at this stage. In some cases fresh evidence of thromboembolism was present in the pulmonary vascular bed. These lesions, however, were not specificially related to the sites where the arteritis was located.

Comment

The arteritis present in the lungs in these cases and that previously reported as rheumatic pulmonary arteritis are similar to the lesions described by Braunstein.[4] He noted their similarity to the classical lesions of periarteritis nodosa, as regards morphology, size of vessel involved, predilection for points of bifurcation, and coexistence of acute and healed lesions. According to Zeek,[6] these same points, and, in addition, the absence of eosinophilic leucocytes and





Figs. 4, 5, 6.—Photomicrographs of pulmonary vessels showing healing phase with fibrosis and vascularization of wall; hematoxylin and eosin.

giant cells in the perivascular infiltrate, tend to distinguish this form of arteritis from hypersensitivity angiitis.

The absence of any regular association of the acute and active lesion with clinical or anatomic stigmata of rheumatic fever and the existence of healed lesions in the presence of acute rheumatic fever would militate against consideration of these lesions as a specific consequence of rheumatic fever. The fact that this form of pulmonary arteritis occurred only in association with advanced mitral stenosis and not with other valvular lesions indicates a significant relationship to mitral stenosis. Similar forms of pulmonary arteritis have been noted in other instances in the absence of rheumatic heart disease where pulmonary hypertension was secondary to such conditions as pulmonary emphysema and congenital heart disease. In advanced mitral stenosis a marked degree of pulmonary hypertension often develops. It is well established that in the accelerated form of systemic hypertension there is frequently present necrotizing arteritis. This is believed to be a consequence of the mechanical effects of the severe hypertension. Waters [7] has also demonstrated that temporary elevations of blood pressure in dogs induced by epinephrine will produce a necrotizing lesion in their coronary arteries. In Ferguson's [5] experiments, in which systemic vessels were anastomosed with the pulmonary circulation, significant pulmonary

hypertension developed with the appearance of acute pulmonary arteritis. Upon subsequent disconnection of these anastomoses, the pulmonary hypertension came down and the arteritis healed. These healed lesions resembled very closely the scarred vessels presently under discussion.

The question naturally occurs as to why this form of arteritis is not seen more frequently in such conditions as pulmonary emphysema, in which pulmonary hypertension is so frequent. Even in advanced emphysema the pulmonary arterial pressure usually does not reach those levels that may occur, at least for temporary periods, in advanced mitral stenosis. The subsidence of these transient high pressures to a more moderate elevation may then permit the lesions to proceed into the healing phase. This might be referred to as the "malignant" phase of pulmonary hypertension, and in a sense it is similar to the accelerated form of systemic hypertension that is often associated with necrotizing arteritis in the kidney.

The differentiation of the healed lesions from organized thrombi is difficult, particularly in those cases in which obvious sources for emboli are present. However, the focal nature of the scarring in the vessel walls and the intramural vascularization favor a previous arteritis rather than the organization of thromboemboli.

It has been mentioned that the few isolated examples of extrapulmonary vascular involvement differ from the pulmonary lesions in several important aspects. Furthermore, according to Zeek,[6] rheumatic arteritis is always associated with clean-cut clinical and anatomic evidence of active rheumatic fever. The rheumatic fever is usually fulminant. Rheumatic aortic arteritis is often present. In the cases studied this was true of this systemic form but not of the pulmonary lesions. In only one instance was systemic arteritis seen in association with pulmonary involvement. This rarity would also be evidence against a similar pathogenesis for the systemic and the pulmonary arteritis.

General Summary

A review of 26 cases in which necrotizing pulmonary arteritis was present in rheumatic heart disease revealed that the arteritis was found only in association with advanced mitral stenosis. The arteritis was present in active and healed states. The morphology of their lesions were quite similar to that seen in polyarteritis nodosa and was differentiated from hypersensitivity angiitis. The lesions were similar to those seen in other conditions in which pulmonary hypertension was produced experimentally. In the absence of advanced mitral stenosis the lesion was never seen in rheumatic heart disease. Therefore the arteritis was considered to be a manifestation of pulmonary hypertension rather than a specific effect of rheumatic fever.

Conclusions

Pulmonary hypertension consequent to the advanced mitral stenosis rather than to specific rheumatic infection is considered to be basic to the pathogenesis of this form of pulmonary arteritis.

The occasional occurrence of isolated systemic arteritis in cases of rheumatic heart disease is considered to be unrelated to the development of the pulmonary arteritis.

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Pancreatic-Acinar Ectasia

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While reviewing the histologic sections of the pancreas from 358 consecutive necropsies on adults for a survey of pancreatitis, we noted a high incidence in pancreatic-acinar ectasia. In 1948 Bagenstoss [1] found that almost half of a series of 270 uremic patients presented these pancreatic changes at necropsy but only 20% of the control series showed such alterations. Warren and Sommers [2] reported that in a series of 60 autopsies on patients with ulcerative colitis 75% revealed these ectatic changes, which they termed pancreatic dystrophy, while only 41% of a control series of 100 revealed similar changes. Although many other types of pancreatic alterations, such as inflammatory-cell infiltration, acinar atrophy, and necrosis, were observed, Ball, Bagenstoss, and Bargen [3] found that 53% of 86 necropsies on patients with ulcerative colitis and 19% of an equal control series had pancreatic-acinar dilatation. In addition, experimental and human studies * on malnutrition, particularly protein deficiency, also have revealed similar changes in the pancreatic acini. Since acinar ectasia is at present only a histologic entity, clinical symptoms could not be correlated with pancreatic-acinar ectasia. However, our observations on this entity suggest that pancreatic-acinar ectasia is a distinct morphologic entity which may progress to focal or, rarely, diffuse parenchymal scarring and may result in subclinical pancreatic deficiency.

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* References 4-5.

Twenty-one per cent of the pancreases studied in 358 consecutive necropsies showed focal or diffuse zones of acinar ectasia. No gross alterations of the organ could be correlated with these microscopic findings. Histologically, different stages in the development of acinar ectasia indicated that this lesion can and does progress to parenchymal scarring. The initial lesion appeared to be the accumulation and inspissation of secretion within the acinus (Fig. 1). The secretion plugs were dense, eosinophilic, and laminated (Fig. 2). Early, a few neutrophiles might also be found within the acinus. With accumulation of secretion within the acinus the parenchymal cells underwent gradual progressive pressure atrophy. The normal pyramidal epithelial cells became flattened and eventually disappeared. In conjunction with these epithelial changes the surrounding stroma became congested, edematous, and infiltrated with inflammatory cells. Initially the cellular infiltrate was composed of neutrophiles, but these were soon replaced by lymphocytes. Occasionally the acinar cells about inspissated secretion showed acute necrosis.

With loss of the acinar cells a collapse type of fibrosis occurred (Fig. 3). The ductules showed no evidence of epithelial metaplasia, luminal obstruction, or inflammation of their walls. In addition, there was no correlation between the degree of arterial or arteriolar disease and pancreatic-acinar ectasia. Because of the loss of acinar tissue and resulting collapse fibrosis, there was an apparent increase in fibrous stroma, infiltrated by a few lymphocytes, and a relative increase in the number of normal-appearing ductules.

Although the cause of these pancreatic alterations is not clear, the morphology suggests that the primary difficulty is the accumulation and inspissation of viscid

secretions within the acinus. Physiologists have shown that reduced volume, increased viscosity, and high enzymatic and protein content of pancreatic secretions can be produced by vagal and sympathetic stimulation, depression of secretin activity, dehydration, and the administration of deficient diets.

The wide variation of primary diseases with which these pancreatic changes may be associated suggests a common basic disturbance in fluid and nutritional balance. However, just as chlorpromazine (Thorazine) [6] or methyltestosterone may, in susceptible patients, produce intrahepatic biliary obstruction by increasing the viscosity of bile, unknown drugs in susceptible patients may also alter the viscosity of pancreatic secretions and produce intra-acinar obstruction.

Review of the necropsy protocols indicated that this histologic entity could not be correlated with any major anatomical cause of death. Neither age nor sex played a role. All of these patients had a malignant tumor, a vascular disturbance with resultant ischemic necrosis or shock, or a severe infection, or a combination of these findings.

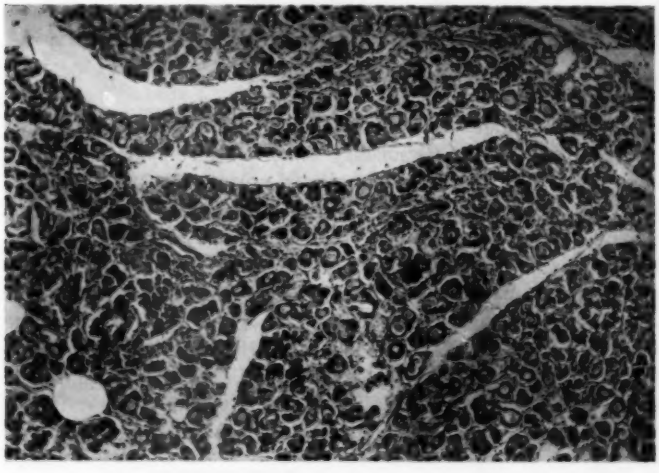

Fig. 1.—Foci of pancreatic-acinar dilatation with accumulation of inspissated secretion. Reduced to 62% of mag. × 120.

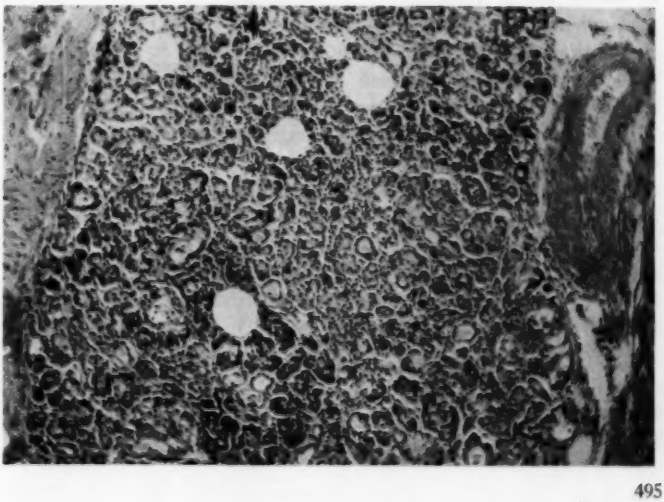

Fig. 2.—Foci of acinar ectasia with focal stromal edema and inflammatory-cell infiltration. Reduced to 62% of mag. × 120.

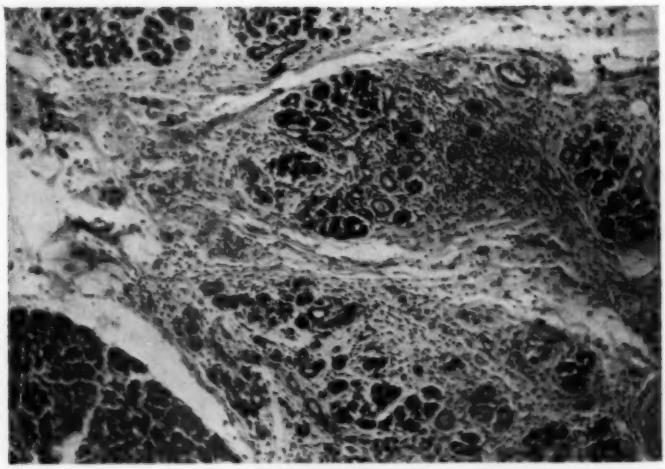

Fig. 3.—Focal loss of pancreatic acini with increase in fibrous stroma and inflammatory-cell infiltration. The ductules are not remarkable. Reduced to 62% of mag. × 120.

Most of these patients survived for a sufficient length of time to demonstrate fluid and nutritional imbalance.

We believe that pancreatic-acinar ectasia, focal or diffuse, is a morphologic expression of a common metabolic disturbance in water balance and/or nutrition secondary to many types of primary illnesses.

Summary

Focal or diffuse ectasia of the pancreatic acini was observed in 21% of 358 consecutive necropsies on adults. The development of acinar ectasia could be traced from its inception to its termination as a minute parenchymal scar. At first there was an accumulation of inspissated secretion within the lumen of the acinus, which was followed by progressive atrophy of the acinar cells. The supporting stroma was congested, edematous, and infiltrated with inflammatory cells. With the disappearance of the acinar cells the lobules became smaller and collapse fibrosis occurred. The ductules were not involved in this process. The end-result was a focus of parenchymal scarring, in which there were a loss of acini, an increase in fibrous stroma, infiltration with chronic inflammatory cells, and an apparent increase in the number of normal-appearing ductules.

The clinical implications of this entity are not clear. However, it is apparent that focal or diffuse parenchymal scarring, the end-result of acinar ectasia, may occur in many different illnesses. It is suggested that a metabolic disturbance in fluid balance and/or nutrition in a number of different diseases may be the common factor in the development of pancreatic-acinar ectasia.

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Histochemical Identification of Chicken Gonadal Cells in Tissue Culture

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The purposes of this study were (1) to identify growing cells in tissue cultures of gonads of chicken embryos by comparing their histochemical reactions with those of cells in the control, or uncultured, gonads, (2) to evaluate the facultative change in enzymatic processes that may take place when cells are cultured, and (3) to determine the histochemical characteristics of gonads of chicken embryos. This study was undertaken because of our interest in the possible teratogenic effect of zinc upon cultured chicken germ cells. In tissue culture, the forms of growing cells were so changed from the usual tissue forms that it became mandatory to seek other means for their identification.

Inasmuch as the techniques which have been employed here may be applied to other problems, it is hoped that these studies in histochemical comparisons may be of general interest.

Materials and Methods

Gonads were obtained from 16- to 21-day chicken embryos. Ovaries and testes were cultured for from two to seven days, depending upon the time required for the appearance of good new growth zones. Tissue cultures were grown in plasma clots either on cover slips or in Porter flasks, with an overlying medium composed of 2 parts Hank's balanced salt solution, 2 parts horse serum, and 1 part chicken embryo juice EE50.

The histochemical techniques used in this study were well-established ones capable of demonstrating alkaline and acid phosphatases, nonspecific esterase, deoxyribonucleic acid, ribonucleic acid, fat, and periodic-acid-Schiff reactions.

A modified Gomori calcium-cobalt technique[1] and Pearse's modified coupling azo dye method[2] were the two methods used for demonstrating alkaline phosphatase. When the latter method was used, the concentration of substrate was increased, as suggested by Burton.[3] Gonads were fixed for from two to three hours in 10% cold neutral formalin in 0.85% sodium chloride solution and were later sectioned upon a CO_2-freezing microtome. Tissue cultures were fixed for from 15 to 30 minutes in 10% cold neutral formol-saline, or for 15 minutes in the same fixative at room temperature. All tissue sections, as well as cultures, were incubated at room temperature for 20 minutes in Michaelis' barbital (Veronal) acetate buffer solution (pH 9.2), containing 0.2 mg. of Na-α-naphthyl phosphate and 3 mg. of Fast Blue Salt BN (General Dyestuff Co.) per milliliter. The solution was filtered onto the slides, and, after staining, the tissue sections were mounted in glycerin jelly. Controls were similar tissues fixed and processed in a manner identical with the test procedure except that the Na-α-naphthyl phosphate was omitted; in a few instances, controls were prepared by inactivating the enzyme with heat.

Burton's[4] technique for acid phosphatase was used, but quantities were changed to 0.2 mg. of Na-α-naphthyl phosphate and 3 mg. of Fast Blue Salt BN per milliliter of 0.1 M barbital acetate buffer of pH 5.8. Tissue blocks and cultures were fixed in cold neutral formol-saline for 15 minutes to 1 hour. Then cover-slip preparations of tissue cultures and frozen sections of tissues were incubated for two hours in the dye-substrate medium at 37 C. Preparations without substrate served as controls.

Gomori's[1] α-naphthyl technique for the demonstration of esterase was used. Tissues were fixed in cold neutral formol-saline, and frozen sections were prepared. Controls were similar to the test

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From the Department of Pathology, Duke University School of Medicine, and the Veterans Administration Hospital.

slides except for the elimination of the α-naphthyl acetate.

The methyl green-pyronin stain for ribonucleic acid (RNA) was performed as outlined by Pearse,[8] but the relative concentration of pyronin was decreased considerably before satisfactory results were obtained. Tissues were fixed in Carnoy's fluid and stained for five minutes. Control sections and cultures were incubated for one hour at 37 C in a 1:100,000 solution of crystalline ribonuclease in distilled water and stained with methyl green-pyronin. Only material removed by ribonuclease digestion was interpreted as ribonucleic acid.

Deoxyribonucleic acid was demonstrated by the Feulgen reaction as outlined by Lillie.[6] Gonads were fixed in Helly's and Carnoy's fluids and in neutral formol-saline. Tissue-culture staining procedures were varied slightly, the cultures being fixed one hour in Carnoy's fluid, treated with 1 N HCl for 10 minutes at 60 C, and left in the Schiff reagent for one-half hour.

For fat stains, all tissue was fixed in neutral formol-saline. Cultures and tissue frozen sections were stained with Sudan IV, counterstained with hematoxylin, and mounted in glycerin jelly.

The staining technique for the periodic-acid-Schiff reaction was essentially the one described by Lillie.[6] Regaud's, Helly's, Carnoy's, and Bouin's solutions, as well as neutral formol-saline, were the fixatives employed. The tissues and some cultures were embedded in paraffin for sectioning, whereas other cultures were stained without sectioning.

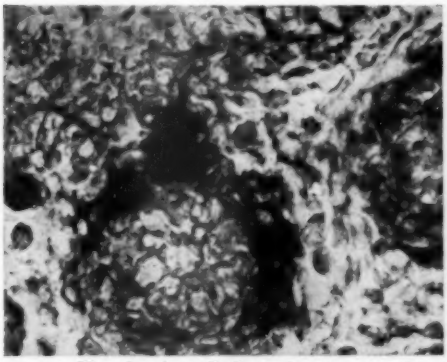
Results and Comment

Results of the various histochemical reactions in tissue sections and in cultures are listed in the accompanying Table and are described in the legends of the photomicrographs.

Cytochemical Reactions of Chicken Embryo Gonads and Tissue Cultures*

	Alk. Phospha-tase	Acid Phospha-tase	Esterase	RNA	DNA	Fat	PAS
Testis tissue							
Germ cell		+	+	++	+		+
Sertoli cell		+++	+++	+	+		++
Basement membrane	+			±	+		+++
Basement membrane cell	++			±	+	+++	+
Indifferent interstitial area cells				+	+		
Leydig cells				±	+	+++	
Capsule cells				±	+		
Endothelial cells				±	+		
White blood cells			+	±	+		+
Testis culture							
Broad fusiform and polyhedral cells		+	+	+	+	+	+
Narrow fusiform cells			+	±	+	+	+
Large round cells				+++	+	++	++
White blood cells				±	+	+	+
Ovary tissue							
Outer germinal cell layer			+++	++	+		+
Germ cells			++	++	+		+
Lipoid cells				±	+	+++	
Endothelial cells				±	+		
Leucocytes				±	+		+
Nonspecific portions of medulla	+		+	+	+		+
Ovary cultures							
Round germ cells overlying new growth			++	+	+	+	++
Thick fusiform cells				±	+	+	
Thin fusiform cells				±	+	+	
Large round cells (degenerate)			++	+++	+	++	

* All reactions are cytoplasmic except for nucleolar RNA and nuclear DNA.



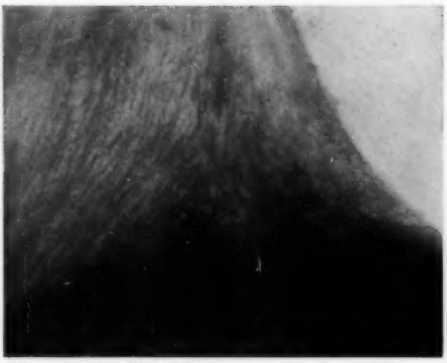
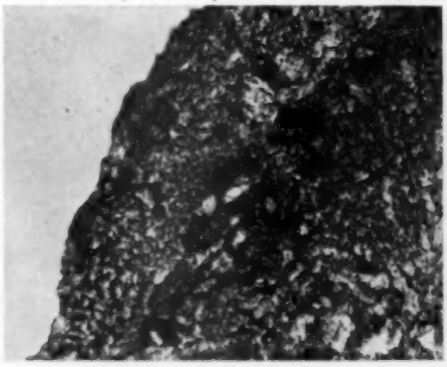


Fig. 1.—Azo-alkaline phosphatase. Sixteen-day testis. Note dark clumps of alkaline phosphatase in peritubular cells. Reduced to 73% of mag. × 900.

Fig. 2.—Azo-alkaline phosphatase. Nineteen-day testis culture. Note dark alkaline phosphatase in explant but absence of alkaline phosphatase in fusiform cells of new growth zone away from explant. Reduced to 73% of mag. × 200.



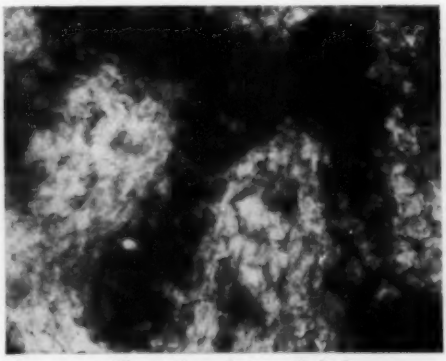


Fig. 3.—Azo-alkaline phosphatase. Seventeen-day ovary. Note only a few small dark areas of alkaline phosphatase activity immediately beneath the germ cell layer. New growth in ovarian cultures contained no alkaline phosphatase. Reduced to 73% of mag. × 300.

Fig. 4.—Azo-acid phosphatase. Seventeen-day testis. Cytoplasm of all tubular cells contains abundant acid phosphatase (dark). Note absence of acid phosphatase in nuclei. Reduced to 73% of mag. × 700.

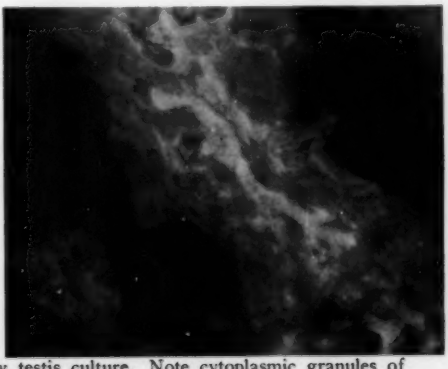
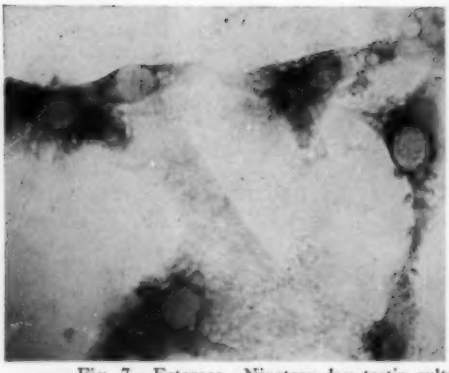


Fig. 5.—Azo-acid phosphatase. Nineteen-day testis culture. Note cytoplasmic granules of acid phosphatase in fusiform cells of new growth zone. Reduced to 73% of mag. × 900.

Fig. 6.—Esterase. Sixteen-day testis. Note that all tubular cells (dark) have much esterase, with relatively less in cytoplasm of germ cell (arrow) and in nuclei. Reduced to 73% of mag. × 1250.



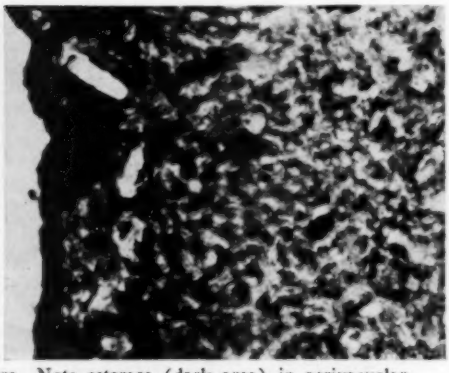
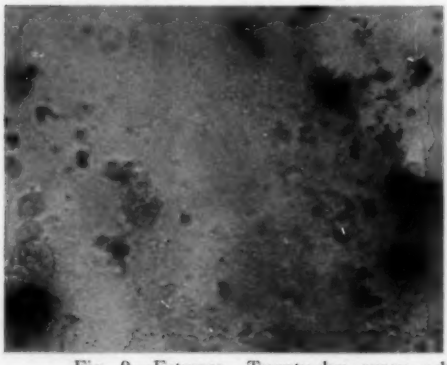


Fig. 7.—Esterase. Nineteen-day testis culture. Note esterase (dark area) in perivacuolar regions of fusiform cells. Reduced to 73% of mag. × 1000.

Fig. 8.—Esterase. Seventeen-day ovary. Note dark esterase in surface germinal layer and in masses of germ cells beneath it. Right side of photo is medullary zone, which has no esterase. Reduced to 73% of mag. × 300.



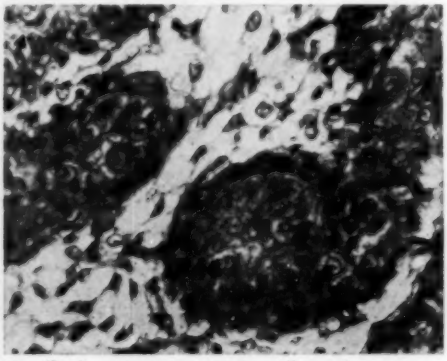
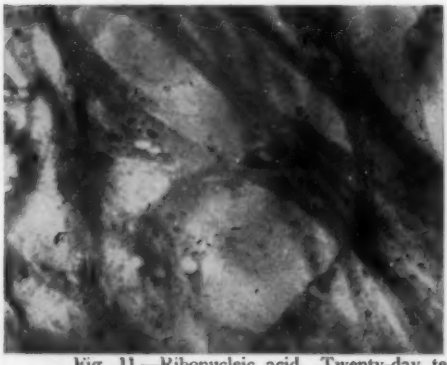


Fig. 9.—Esterase. Twenty-day ovary culture. Dark masses are germ cells with very positive reaction for esterase. Reduced to 71% of mag. × 700.

Fig. 10.—Ribonucleic acid. Twenty-day testis. Note diffuse staining of cytoplasm of all tubular-peritubular cells and paranuclear stain of germ cells (arrow)., Reduced to 72% of tubular and peritubular cells and paranuclear stain of germ cells (arrow). Reduced to 72% of mag. × 900.



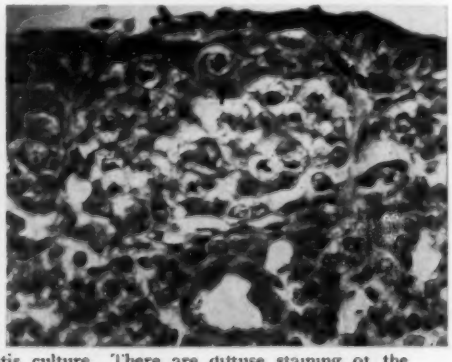


Fig. 11.—Ribonucleic acid. Twenty-day testis culture. There are diffuse staining of the cytoplasm and prominent nucleoli in fusiform cells. Reduced to 72% of mag. × 900.

Fig. 12.—Ribonucleic acid. Twenty-day ovary. Upper layer is germ cells; lower, medullary cells. Note fine cytoplasmic stippling, paranuclear caps (arrows), and prominent nucleoli in germ cells. Reduced to 72% of mag. × 900.

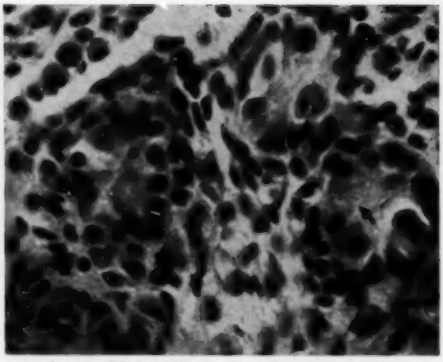
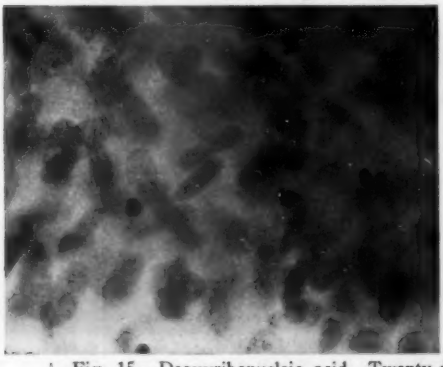


Fig. 13.—Ribonucleic acid. Twenty-day ovary culture. Note diffuse cytoplasmic stain in fusiform cells in upper right and dark cytoplasmic clumps of ribonucleic acid in round germ cells in left center. Reduced to 72% of mag. × 900.

Fig. 14.—Deoxyribonucleic acid. Twenty-day testis. Tubules have germ cells with coarsely clumped chromatin (arrows). Adjacent peritoneal cells have more finely dispersed ribonucleic acid. Reduced to 72% of mag. × 1300.



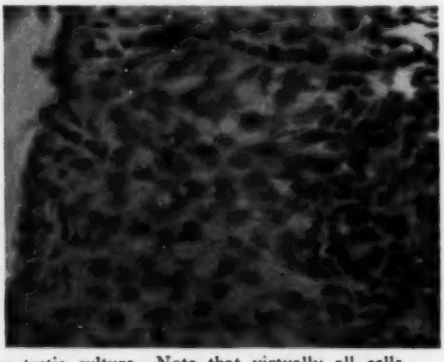


Fig. 15.—Deoxyribonucleic acid. Twenty-day testis culture. Note that virtually all cells in new growth zone have finely dispersed ribonucleic acid. Reduced to 72% of mag. × 900.

Fig. 16.—Deoxyribonucleic acid. Twenty-day ovary. Left portion is germ cell layer, with prominent clumping of germ cell nuclear chromatin, as contrasted with medullary cell layer on right. Reduced to 72% of mag. × 900.

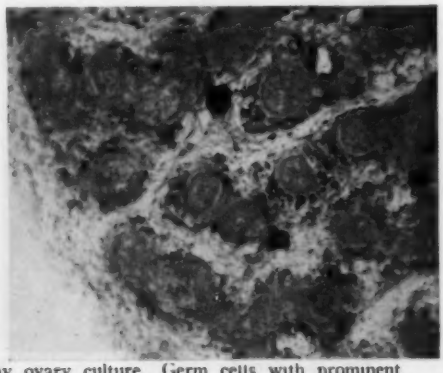
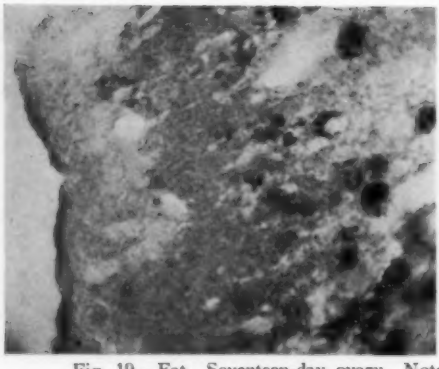


Fig. 17.—Deoxyribonucleic acid. Twenty-day ovary culture. Germ cells with prominent chromatin and spiremes are seen at the margin of the culture. Reduced to 72% of mag. × 1180.

Fig. 18.—Fat. Twenty-one-day testis. Note fat (dark) in peritubular cells and in round masses in interstitial area. Reduced to 71% of mag. × 200.



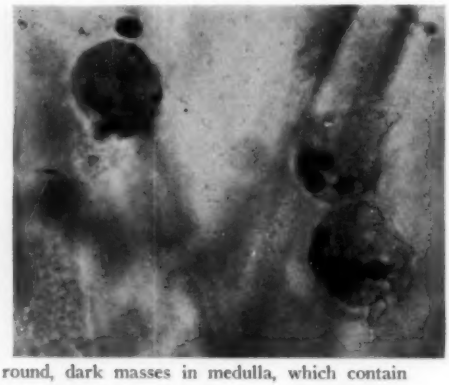
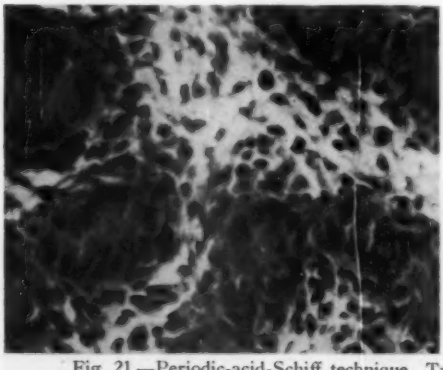


Fig. 19.—Fat. Seventeen-day ovary. Note round, dark masses in medulla, which contain much fat. Reduced to 71% of mag. × 200.

Fig. 20.—Fat. Seventeen-day testis culture. Note large round cells with fat (dark)-containing vacuoles. Reduced to 72% of mag. × 1180.



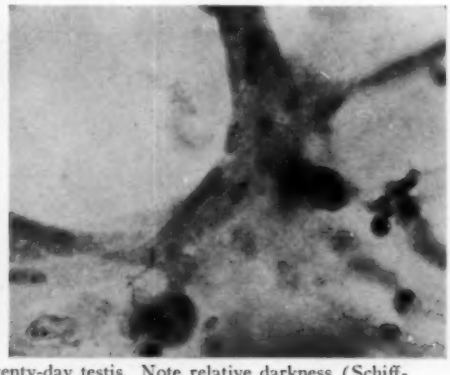
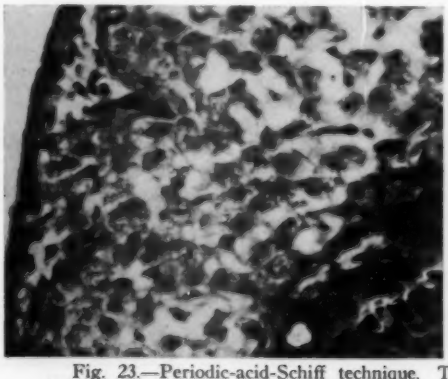


Fig. 21.—Periodic-acid-Schiff technique. Twenty-day testis. Note relative darkness (Schiff-positive material) of basement membrane and all tubular cells. Reduced to 72% of mag. × 900.

Fig. 22.—Periodic-acid-Schiff technique. Sixteen-day testis culture. Note dark granules of Schiff-positive material in large round cells. Reduced to 72% of mag. × 900.



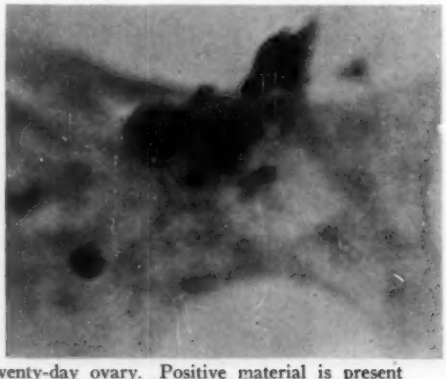


Fig. 23.—Periodic-acid-Schiff technique. Twenty-day ovary. Positive material is present in the outer germinal layer, the germ cells, and the medullary cells, in cytoplasm and nuclei. Reduced to 72% of mag. × 900.

Fig. 24.—Periodic-acid-Schiff technique. Sixteen-day ovary culture. Round germ cells contain positive material in cytoplasm and nuclei. Reduced to 72% of mag. × 900.

Most cells in routine histologic sections of chicken embryo gonads, including germ cells, primitive Sertoli cells, red and white blood cells, endothelial cells, interstitial cells of Leydig, tubule basement membrane cells, ovarian stromal fat-bearing cells, and interstitial and capsular connective tissue cells were identified readily by their morphology. Similarly, in tissue cultures of chicken embryo gonads there were several easily identifiable morphologic cell types, such as white blood cells, red blood cells, some endothelial cells, and migrating ovarian germ cells. Other cells, such as broad polyhedral and broad fusiform and narrow fusiform cells, were not easily identified and might have been of fibrous, germ, or Sertoli cell origin.

Several histochemical features were of aid in identifying the broad fusiform and polyhedral cells in tissue cultures as germ cells. For instance, both tissue germ cells and tissue-culture germ cells, such as migrating ovarian and testicular germ cells, and broad polyhedral and fusiform cells were demonstrated to have cytoplasmic Schiff-positive material, esterase, and acid phosphatase, as well as relatively larger amounts of RNA than other cells. The migrating ovarian germ cells and a few loose testicular germ cells scattered in the new growth zones could be identified clearly by their prominent, clumped nuclear chromatin, visible in hematoxylin and deoxyribonucleic acid preparations. However, the nuclei of the broad fusiform cells in both testicular and ovarian cultures were so altered by their adaptation to culture that their positive identification as germ cells on the basis of distinctive nuclear chromatin arrangement was not possible. The alkaline phosphatase reaction has been reported to be of value in identification of primordial germ cells in the mouse embryo,[6] but in our studies no alkaline phosphatase was observed in the control chicken tissue sections or in tissue cultures bearing germ cells.

The Sertoli-cell antecedents in the embryo testes, the peritoneal cells, contained a minimum quantity of ribonucleic acid, but somewhat more Schiff-positive material, and had an abundant esterase and acid phosphatase content. The thin fusiform cells in cultures of embryo testes contained esterase. The acid phosphatase and RNA reactions in tissue cultures were so minimal that it was hardly possible to identify either substance in the thin fusiform cells.

Although the germ cells and peritoneal cells could be distinguished easily in the testis tissues without histochemical aids, it was difficult to separate their tissue culture counterparts even by using both morphologic and histochemical criteria. Morphologically, the supposed germ-cell counterparts in the new growth zone of tissue cultures had an abundance of cytoplasm in either plump polyhedral or broad fusiform shape, and a paranuclear, cap-like distribution of mitochondria, which is also characteristic of germ cells in tissue sections. The supposed Sertoli-cell counterparts possessed only a thin fusiform shape and did not show any characteristic distribution of mitochondria. Histochemically, the broad fusiform and polyhedral (germ) cells contained Schiff-positive material, esterase, acid phosphatase, and RNA. The thin fusiform (Sertoli) cells contained Schiff-positive material, but little or no esterase, acid phosphatase, or RNA. In contrast, both germ and peritoneal cells contained acid phosphatase, esterase, and RNA in tissue. The primitive tissue Sertoli cells contained more acid phosphatase and esterase, whereas the germ cells contained more RNA. Because of minimal reactions in tissue culture, it was not possible to differentiate germ cells from peritoneal cells by means of comparison of esterase and acid phosphatase reactions, but the relative abundance of RNA in tissue germ cells and their culture counterparts, broad fusiform cells, does corroborate the impression that the broad fusiform cells are of germ cell origin.

Certain connective tissue elements, such as the peritubular layer of cells and asso-

ciated basement membrane in the embryo testis and rare foci of fusiform cells in the medullary portion of the embryo ovary, contained alkaline phosphatase. However, no alkaline phosphatase activity was seen in tissue cultures, indicating that the tubule basement membrane cells contribute minimally, if at all, to the new growth zone in gonad tissue cultures.

In the chicken testes, the interstitial cells contained an abundance of fat, as did masses of medullary lipoid cells in the embryo ovary. By using the fat stains on tissue cultures, it was not possible to tell whether any Leydig cells or ovarian lipoid cells were present, since most cells in culture developed fat-positive vacuoles at some time during their life. The fatty vacuolation noted in cultured cells was probably indicative of an imperfect nutrient and physical environment, since many cells in the new growth zone showed little or no vacuolation in the first few days of growth.

Thus, the histochemical composites of the various types of cells in tissues remained in these cells and their progeny as they metabolized in tissue culture. For instance, the Schiff-positive carbohydrate present in germ cells in tissues was found also in cell variants of germ cells in tissue culture. However, there was relatively less ribonucleic acid, esterase, and acid phosphatase in fusiform cells in culture than in their cells of origin in tissues, the germ and peritoneal cells. This difference suggested a quantitative change in the cell content of RNA, esterase, and acid phosphatase caused by tissue culture. The physical state of cells in tissue culture was a flattened and spread-out one, as compared with the counterpart of these cells in tissue; this thinness of cytoplasm may account for some of the apparent decrease of RNA, esterase, and acid phosphatase in culture.

Summary and Conclusions

In order actually to observe possible teratogenic changes in germ cells produced by zinc, it is necessary to culture germ cells and to identify these germ cells in the new growth zone of tissue cultures. In an attempt to identify cells by histochemical means, a number of representative tissue sections and tissue cultures were prepared from gonads of 16- to 21-day chicken embryos. Both sections and cultures were then tested histochemically for alkaline phosphatase, acid phosphatase, esterase, ribonucleic acid, deoxyribonucleic acid, fat, and periodic-acid-Schiff-positive material, in an effort to identify specific types of cells in the tissue cultures.

From these studies it may be concluded that (1) histochemical tests aid in the identification of germ cells and other untyped cells in tissue cultures when histochemical reactions of gonadal tissue and tissue cultures of chicken embryos are compared, (2) alterations of cell metabolism and structure arising through tissue culture are quantitative rather than qualitative, and (3) information relating to the metabolism of the various cells in tissues and in tissue cultures may be obtained from the histochemical tests used in this study.

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Laboratory Methods and Technical Notes

A RAPID MALLORY TRICHROME STAIN (CHROMOTROPE-ANILINE BLUE)

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and
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Paterson, N. J.

The name trichrome stain is applied to a mixture of one of the sulfonated azo dyes with an acid triphenylmethane dye in an acid medium.[1] Such mixture, in combination with a metallic mordant (phosphotungstic or phosphomolybdic acid), is capable of coloring in a differential manner a variety of tissue elements—connective tissue fibers, basement membranes, myofibrils, cytoplasmic granules, and various colloids and hyalins. The stain exists in numerous modifications, both elaborate and simple—Mallory, Heidenhain, Masson, Gomori, and Gallego,[2] to name only a few. Aniline blue, light green, and fast green are the most commonly used triphenylamine dyes. As the azo dye of choice Gomori[1] recommended chromotrope 2R, C.I. No. 29. Recent studies by Roque[3] and by Wagner[4] have shown that the uptake of the dyes (particularly the azo dye) by the tissues is at its maximum in a comparatively narrow and strongly acid pH range (2.0-2.2). Based upon these considerations, a simple and rapid procedure was adopted which is a good general trichrome stain and is particularly helpful in the study of fine fibers and hyalins.

Procedure

For best results, tissues should be fresh and well fixed. Postmortem autolysis is deleterious, and overfixation, particularly in acid solutions, should be avoided. Any fixative can be used, but the resulting color schemes vary and are not necessarily comparable. Fixatives containing mercury give good results, Helly's fluid being preferable to Zenker's. Neutral buffered formalin is very satisfactory, particularly if the sections are mordanted before staining in saturated mercury bichloride U.S.P. for two to six hours at 56 C, or overnight at room temperature; mercury precipitate is removed in the usual manner, with iodine and thiosulfate. After any fixation, weak red shades can be enhanced by mordanting before staining in warm (45-50 C) Bouin solutions for two to five minutes and washing sections in running water until the yellow color disappears.[1]

1. Stain the nuclei for two to three minutes in acidified crystal violet (0.1% crystal violet in 0.2% acetic acid). Differentiate for 30-45 seconds in 0.2% acetic acid. Rinse well in distilled water.
2. Mordant 30-45 seconds in 1% aqueous solution of phosphomolybdic acid. Rinse well in distilled water.
3. Stain two minutes in chromotrope 2R 1 gm., aniline blue 0.25 gm., 0.02 N hydrochloric acid 50 ml. (Dissolve aniline blue in the acid with the aid of gentle heat; cool; add chromotrope 2R. Solution keeps well for many months in a Coplin jar.)
4. Rinse in water and dehydrate quickly through 95% and absolute alcohols, or more slowly through three changes of tertiary butyl alcohol 3 parts, absolute ethyl alcohol 1 part. Clear in xylene and mount.

Results

In formalin-fixed material, nuclei stain purplish; cytoplasm, pale-blue to pink; muscle, red; collagen, reticulum fibers, and basement membranes, brilliant blue; fibrin and serum protein, red; red blood cells, "fibrinoid," keratin, bright red; colloids and hyalins, blue to purple to red. After premordanting in mercury bichloride, the elastic fibers and mucus stain purple.

Submitted for publication June 27, 1956.
From the Laboratories, Barnert Memorial Hospital.

Comment

In tissues stained with any trichrome method, one can distinguish three types of structures: 1. Those that stain intensely with the triphenylmethane dye (blue or green)—collagen fibers, reticulum fibers, and basement membranes. 2. Those that stain strongly with the sulfonated azo dye (red or orange)—red blood cells, keratin, some "fibrinoids." 3. Those that have weak affinity for either dye—nearly all other basic tissue elements.

Structures in Groups 1 and 2 bind the appropriate dye quite independently of the type of fixation and, to a great extent, independently of the ratio of the dyes in the staining solution, the presence or absence of mordants, or even the pH of the solution, provided the last remains distinctly acid. Roque[3] has demonstrated that in vitro only glycoprotein bound aniline blue, while other proteins tested stained with chromotrope 2R. While these results cannot be directly transposed to staining of tissue sections, strong affinity for aniline blue in the presence of certain metals may very well represent a histochemical test for some types of glycoproteins. The common denominator of the diverse proteins in Group 2, showing marked affinity for chromotrope 2R, seems to be their strongly basic character.

The staining of materials in Group 3 is markedly affected by postmortem autolysis, the choice and duration of fixation, and the pH of the staining solution. In the absence of a mordant, these materials take on a deep purple hue, indicating the uptake of both aniline blue and chromotrope 2R. The mordants, such as phosphotungstic and phosphomolybdic acids, trinitrophenol, and mercury bichloride, depress the affinity for the former dye (this is in contrast to substances in Group 1) and increase it for the latter. Most consistent results are obtained by double mordanting, first in mercury bichloride and later in phosphomolybdic acid. Under carefully standardized conditions, some of the substances usually stain blue and others, usually red, but there are considerable variations, probably dependent upon the physicochemical state of the protein. Fresh fibrin stains red, but old fibrin is pinkish blue to blue. Colloid of the thyroid gland and the pituitary stains blue in some follicles, red or purple in others; often in a single follicle the periphery is blue and the center red. The hyalin of arteriosclerosis is nearly always bright red, while that of the intercapillary glomerulosclerosis shows all stages between purple-red and blue.

Summary

A simple and rapid trichrome stain is described utilizing the aniline blue-chromotrope 2R mixture at pH 2.0-2.2.

The stain is useful for demonstration of very fine structures (reticulum fibers, basement membranes, myofibrils) and for the study of the physicochemical state of hyalins and colloids.

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News and Comment

PERSONAL NEWS

Capt. Theodore R. Austin Assigned to Great Lakes, Ill.—Capt. Theodore R. Austin, who has been stationed in the Armed Forces Institute of Pathology for the past four and a half years, has been assigned to the U. S. Naval Hospital, Great Lakes, Ill., as chief of the laboratory service.

Dr. E. E. Faulter Associate Professor of Pathology, University of Mississippi.—Dr. E. E. Pautler has been made associate professor of pathology at the University of Mississippi in Jackson.

Dr. Robert S. Jason President of Association of Former Internes and Presidents of Freedmen's Hospital.—Dr. Robert S. Jason, dean and professor of pathology at the Howard University College of Medicine, Washington, D. C., was recently installed as president of the Association of Former Internes and Residents of Freedmen's Hospital.

Dr. Frank W. Hartman Appointed Medical Research Advisor to Surgeon General of U.S. Air Force.—Dr. Frank W. Hartman, of Detroit, has been appointed medical research advisor to the Surgeon General of the U. S. Air Force.

Appointments in Pathology, State University of New York College of Medicine.—Drs. Harold R. Cottle and Philip H. Prose have been promoted to assistant professors of pathology at the State University of New York College of Medicine, New York.

Dr. Sophie Spitz Dies.—Dr. Sophie Spitz, director of the pathology department of the New York Infirmary and assistant professor of pathology at the Sloan-Kettering Division of Cornell University Medical College, died on Aug. 10, in New York City.

Dr. Bernard Steinberg Awarded John A. Hartford Foundation, Inc., Grant.—Dr. Bernhard Steinberg, Director of the Toledo Hospital Institute of Medical Research, has been awarded a grant of $162,075 by the John A. Hartford Foundation, Inc., for a three-year study at the Toledo Hospital of the causes and treatment of leukemia. The investigation will comprise clinical work on patients, utilizing findings already developed in the Institute, and based on the concept that leukemia is a disease in which there is a deficiency of the regulating hematopoietic factor. Associated with Dr. Steinberg in the investigation will be Dr. M. A. Atamer, hematologist, and Dr. Leon Libenson, biochemist.

ANNOUNCEMENTS

Symposium on Fundamental Cancer Research.—The University of Texas M. D. Anderson Hospital and Tumor Institute will hold its 11th Annual Symposium on Fundamental Cancer Research on March 7, 8, and 9, 1957. The topic of the meeting will be "Viruses and Tumor Growth." An advisory committee is now formulating the program for the symposium.

National Library of Medicine.—President Eisenhower, on Aug. 3, signed a bill establishing a National Library of Medicine in the Public Health Service, and transferring to the Service all of the functions of the Armed Forces Medical Library. The exact site for the Library has not been determined, but it will probably be in the Washington area. The policies of the new library will be guided by the advice and recommendations of a Board of Regents, consisting of seven ex-officio members, namely, the Surgeons General of the Public Health Service and the Army, Navy, and Air Force, the Chief Medical Director of the Department of Medicine and Surgery of the Veterans Administration, the Assistant Director for Biological and Medical Sciences of the National Science Foundation, the Librarian of Congress, and 10 additional members appointed by the Senate. The appointive members will serve four years.

Health Research Facilities Program.—On July 30, 1956, the President signed the Health Research Facilities Act of 1956, which authorizes the appropriation of funds not to exceed $30 million for each of three years to assist in the financing of the construction of facilities for research in the "sciences related to health." The Act defines "sci-

ences related to health" as including medicine, osteopathy, dentistry, and public health, and fundamental and applied sciences when related thereto. The assistance is to be in the form of grants in aid to public and nonprofit institutions on a basis of not more than 50% for the Federal share. Items not creditable for matching purposes include (1) costs for the acquisition of land or off-site improvements and (2) obligations made prior to the award of the research facilities grant concerned. The Congress has now appropriated $30 million to the Public Health Service, Department of Health, Education and Welfare, for this program. These funds are now available and will remain available until expended.

The funds are to be used to provide additional research facilities through the construction and/or equipping of new buildings or the expansion, remodeling, alteration, and/or equipping of existing buildings.

The new law provides for the appointment of a National Advisory Council on Health Research Facilities. It is expected that this Council will meet in the near future to establish policies and approve regulations for the administration of the new program. The Surgeon General is not permitted to award a research facilities grant in aid which has not previously been approved by the Council.

Application forms, as well as additional information, will be supplied promptly upon request to the Division of Research Grants, National Institutes of Health, Public Health Service, Bethesda 14, Md.

Books

Biochemical Mechanisms in Inflammation. By Valy Menkin, M.D. Price $9.50. Pp. 438, with 166 figures. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1956.

This monograph is a modernized version of Menkin's views on inflammation. It is an extension and reiteration of the original monograph, published in 1940. "Leukotaxine" and "leukocytosis-promoting factor" have now been joined by "necrosin," and "pyrexin," and "exudin." The role of local pH in conditioning inflammatory-cell type is maintained. Local lymphatic blockade is defended as the important defensive mechanism. The discovery of the anti-inflammatory effect of corticotropin and cortisone is shown to be Menkin's contribution to modern medicine. It is unfortunate that Menkin's contribution to the study of inflammation is beclouded by too strong a bias in favor of the investigator, and not strong enough a bias in favor of Nature. Nature does not concern itself with man's opinions. If one looks around this bias, then one finds some interesting information in the book.

Oral Cancer and Tumors of the Jaws. By George S. Sharp; Weldon K. Bullock, and John W. Hazlet. Price $15.00. Pp. 561, with 647 illustrations. The Blakiston Division, McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 36, 1956.

The authors' purpose in writing this book is to provide a well-illustrated manual for use by the general practitioner and dentist in the recognition of oral cancer and tumors of the jaws.

The text is well organized into five divisions, dealing with introductory principles of neoplasia; tumors, benign and malignant, of the oral mucosa and accessory cavities; neoplasms, benign and malignant, of the jaws; quasitumors of the jaws, and cysts of the jaws.

Each chapter is comprehensive in dealing with most lesions encountered in the specific locale. The lesions are treated briefly by a discussion of the regional anatomy of the area, clinical and histological appearance of the lesion, differential diagnosis, and treatment. The bibliography at the end of each chapter is not extensive, being limited to a few selected references, but is adequate for one seeking additional information.

The emphasis being on clinical manifestations of oral cancer, the authors have illustrated the book profusely with 647 photographs and radiographs, of a high degree of excellence in most instances. There are no reproductions of the histopathology of the lesions described.

This book would make an excellent addition to the library of the clinician who has occasion to see and diagnose oral lesions.

Lehrbuch der speziellen pathologischen Anatomie fuer Studierende und Aertze. By Eduard Kaufmann. Editions 10 and 11. Vol. III, Teil 1. Edited by Prof. Martin Staemmler, D.M. 17.60. Pp. 98, with 27 illustrations. Walter de Gruyter & Co., Genthiner Strasse 13, Berlin W 35, 1956.

The first part of Volume III of the new Kaufmann (Staemmler) "Lehrbuch" deals with diseases of the meninges. Also included are alterations of the blood sinuses and abnormalities of the ventricular system. Written by W. Wepler, it gives detailed information on every phase of this subject. It is clearly written and should not be too difficult to understand for anybody with a reading knowledge of medical German. Unfortunate is the introduction of the term "pachymeningiosis" in the sense of non-inflammatory changes of the dura mater, under which also subdural hematoma is included. This is in spite of the fact that the term nephrosis has been established and the term "myocardosis" is used more and more to denote degenerative rather than inflammatory change. While it is true that the ending "osis" is defined as "a word termination denoting a process, often a disease or morbid process," it also "conveys the meaning of abnormal increase" (The American Illustrated Medical Dictionary, Ed. 22, W. A. N. Dorland, Philadelphia and London, W. B. Saunders Company, 1951, p. 1064).

The more detailed discussion of blood vessel changes occurring in the various meningitides is a very welcome factor, often missing in similar studies. Also, diseases brought by fungi are given in detail. Tumors of the meninges are not included in this part of the volume. As far as references are concerned, it would help if the initials of the authors were included, if the abbreviations of the various journals were uniform throughout, and, if possible, if the full titles of the cited articles could be provided.

Atlas of Exfoliative Cytology, Supplement I. By George N. Papanicolaou, M.D., Ph.D. Pp. 50, with 16 plates in color. Harvard University Press, Cambridge 38, Mass., 1956.

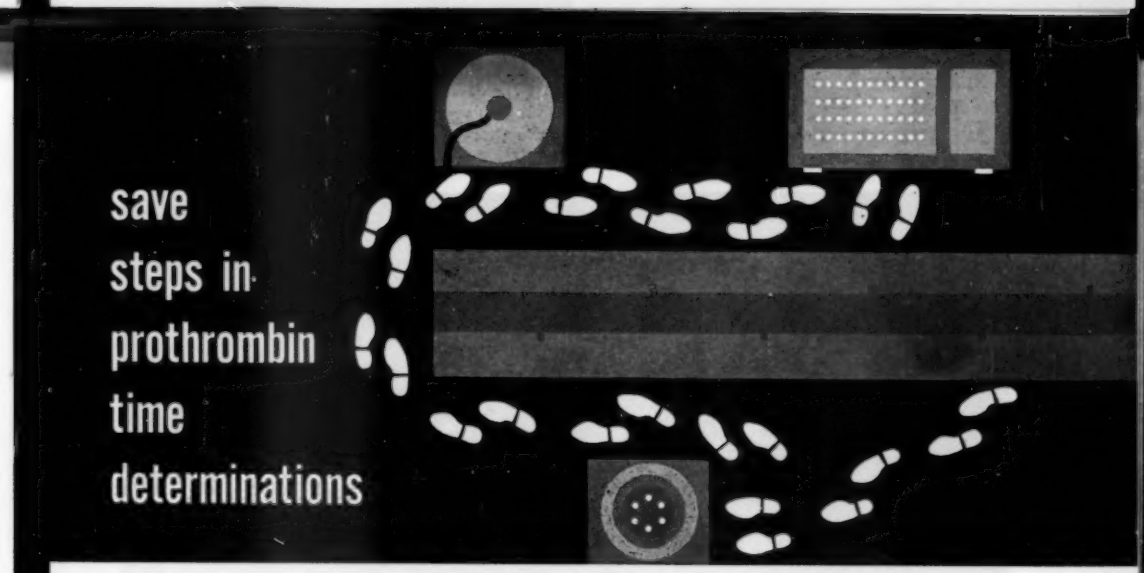
The appearance in 1954 of the "Atlas of Exfoliative Cytology" marked the first time that a comprehensive study of cytologic methods was available to the pathologist. Excellent color plates illustrating various cell types found in cytologic smears were the outstanding feature of this atlas. The original format of the atlas was loose-leaf style, to permit periodic updating of the material by the addition of supplements. The first supplement to the "Atlas" does not so much offer new material or methods as correct what was a deficiency of the original "Atlas." This deficiency, particularly from the pathologist's viewpoint, was the lack of histologic and cytologic correlation. Fourteen of the sixteen plates in Supplement I contain color illustrations of histologic material taken from the same case as the cytologic smears. This conveys much more graphically than text both the similarities and the differences of tumor cells as seen in tissue sections and fluid smears. Often the cytologic preparation reveals the cells to be larger than is apparent in sections. Pyknosis and engulfment of tumor cells are well illustrated in such a way that it is possible to compare directly sectioned and stained material. It is this correlation of histologic and cytologic patterns that is of greatest value to the pathologist.

The present supplement corrects an omission of the original volume and shows cytologic changes secondary to Trichomonas infection. Only two minor criticisms are apparent in this work. It is unfortunate that the author does not emphasize important points in interpretation of cytologic smears. Throughout the discussions essential points of interpretation are lost in general descriptions, or even case histories. One point of possible confusion to the pathologist is the use throughout the supplement of the word "secondary" instead of "metastatic."

The supplement continues the same format as the original atlas, with discussions and descriptions of each of the color plates. The illustrations are excellent, all being in color and the cytologic-smear reproductions having been retouched to improve color qualities. It is apparent that this supplement is going to render the "Atlas of Exfoliative Cytology" of even greater value to the pathologist.

Ionography: Electrophoresis in Stabilized Media. By Hugh J. McDonald. Price, $6.50. Pp. 268. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago, 11, 1955.

This is a very interesting, well-constructed monograph on an important technique in modern biological investigation. The author has had a considerable personal experience with the method. The main technique described is that of paper electrophoresis. The author describes in some detail the apparatus of his own design, which may or may not have the advantages claimed. There is an interesting discussion of the problem of relating the mobilities and concentrations found by migration of protein on paper and staining with dyes and the Tiselius method, using schlieren patterns for measurement of components. Five chapters are devoted to the special characteristics and problems of separation of proteins, peptides and amino acids, carbohydrates, lipoproteins, enzymes, hormones, vitamins, and inorganic substances. Described here are not only the problems in methodology but also many of the investigations carried out in recent years are cited. A final chapter deals with a miscellany of applications of the technique. The bibliography has 760 references. The book is a useful addition to those already at hand on this subject.



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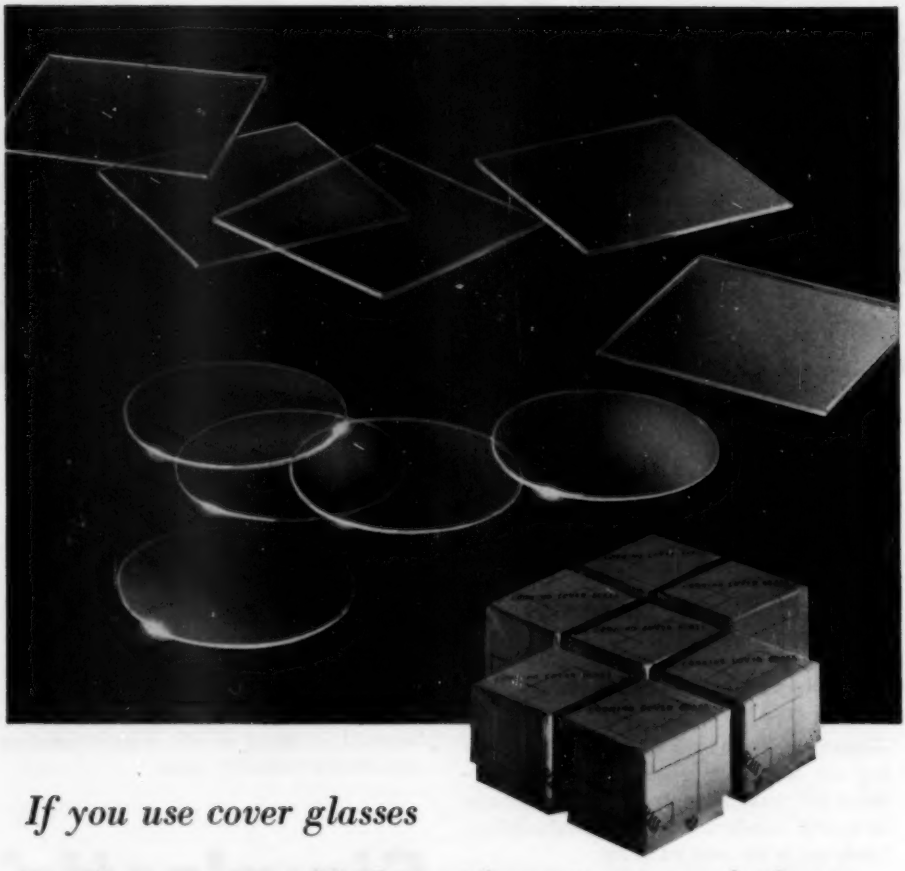

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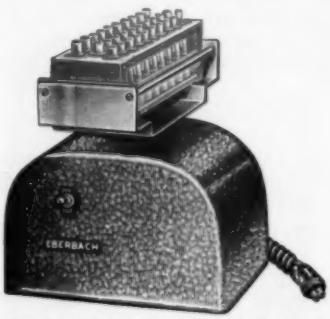

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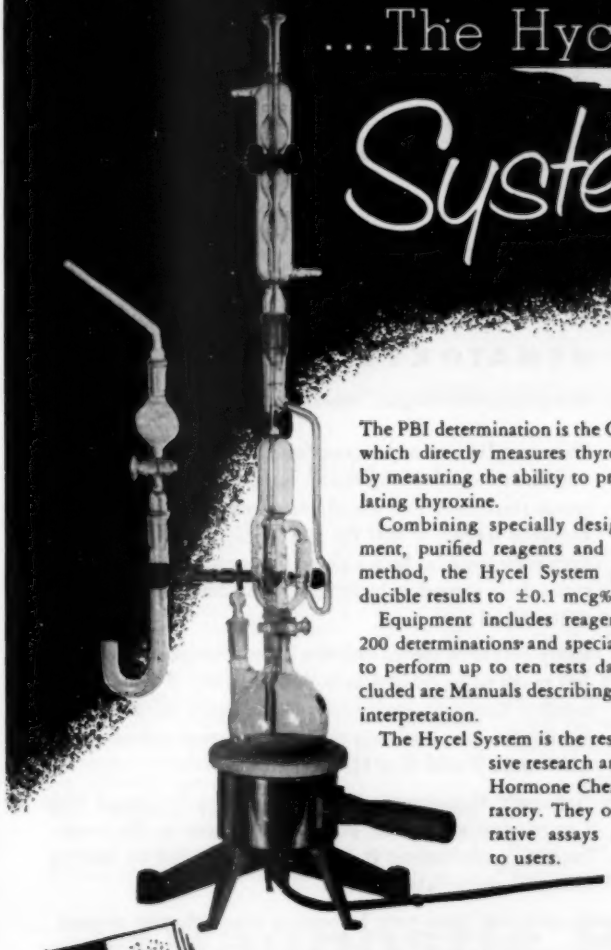

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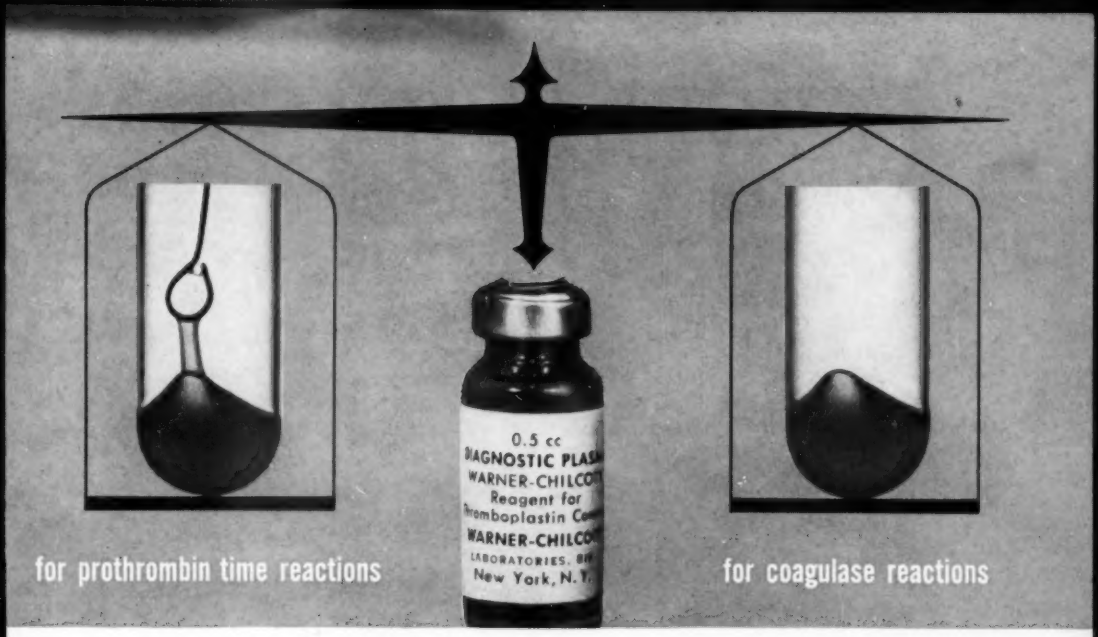

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References: 1. Tager, M.: Conference on Staphylococcal Infections, New York Academy of Sciences (Feb. 16) 1956. 2. Boyd, H.: First North American Conference of Medical Technology, Quebec (June 19) 1956.

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